USING SPECIES DISTRIBUTION MODELLING AND GENETIC ANALYSIS FOR THE CONSERVATION OF RARE SPECIES: CASE STUDIES ON EUROPEAN BATS

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Abstract

This study examines the applicability of species distribution modelling combined with genetic analysis to fill knowledge gaps for poorly documented species, thus giving support to more adequate conservation policies. In this thesis, *Barbastella barbastellus* was the chosen studied species due to its rarity and wide knowledge gap. By comparing the accuracy of two different presence-only modelling techniques (Maximum Entropy (Maxent) and Ecological Niche Factor Analysis (ENFA)), it is shown that Maxent outperformed ENFA by successfully predicting *B. barbastellus* distribution in Portugal, with the discovery of several populations and extending known distribution ca. 100 km further south. In a second phase of this work, Maxent models were updated with new presence records of *B. barbastellus* and also projected to conditions during the Last Glacial Maximum (LGM) to determine the species' palaeo-distribution in Portugal. These results were integrated with mtDNA analysis to show that Portuguese populations constituted a homogeneous conservation unit, despite a predicted isolation between northern and southern populations during the LGM. By widening the geographical scope of this study in order to cover species' range, glacial refugia were identified in the southern European peninsulas of which only Iberia had no contribution for the postglacial colonisation of Europe. Finally, the research focused on the potential impact of future climate change on 28 European bat species, grouped according to their biogeographic patterns. Species associated with colder climates will suffer severe challenges for their survival and major regional extinctions are predicted whichever future climate change scenario is modelled. The future distribution of species associated with warmer climates varied according to the scenario

modelled, and more severe climatic predictions resulted in a general contraction of each species' range. The research covered in this thesis shows that by integrating predictive modelling with genetic analysis it is possible to fill knowledge gaps in rare species, with implications for conservation.

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Hugo Emanuel Vitorino Rebelo

SIGNED: ..... DATE: Bristol, 31st July 2009

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CHAPTER 1

General Introduction

"Read not to contradict and confute,
not to believe and take for granted,
not to find talk and discourse,
but to weigh and consider"

Sir Francis Bacon

1.1 Importance of biodiversity conservation

Biodiversity is a concept which refers to the range of variation or differences within the living world (World Conservation Monitoring Centre 1992). Biodiversity is most widely considered as synonym of species diversity. More recently, genetic diversity is also being taken into consideration when addressing biodiversity conservation issues. The variability of a gene pool at both the levels of species and populations is directly associated with survival. A lack of genetic variability can diminish resistance to diseases while increasing the prevalence of genetic-related maladies, and may also reduce a species' fitness response to natural evolutionary change (Booy *et al.* 2000; Hedrick & Kalinowski 2000; Amos & Balmford 2001; Frankham 2005). Moreover, biodiversity should also comprise the ecosystems and the ecological complexes of which all integrated species are part (Convention on Biological Diversity, http://www.cbd.int/convention/articles.shtml?a=cbd-02; Millennium Ecosystem Assessment 2005).

At present global biodiversity is under threat, raising the alarm of an unprecedented mass extinction as a direct result of human activities, especially from habitat destruction or alteration (Vié *et al.* 2009). Over recent decades several international policies (e.g. Habitats Directive, Convention on Biological Diversity) have been formulated as a response to this problem. Indeed, parties of the Convention on Biological Diversity proposed the fairly ambitious deadline

of stopping biodiversity loss by 2010. The European Council (2001) also committed themselves to this goal. It is clear now that this goal will not be accomplished, like almost all of these conservation measures failed to fully achieve respective goals at a global scale, with an ever increasing number of species attaining threatened status (Vié *et al.* 2009). Nevertheless, the rate of emerging threatened species has apparently slowed down in some temperate regions (World Wide Fund for Nature 2008), where these policies have been more strongly enforced.

1.2 Ecology and conservation of bats

Bats (Order Chiroptera) play a key role in the maintenance of biodiversity levels, constituting almost a quarter of the mammal species (Kunz & Pierson 1994; Simmons 2005). They are the only mammals capable of powered flight, and possess other specialised characteristics (e.g. echolocation, hibernation) that make them excellent subjects for research on evolution (e.g. Teeling *et al.* 2000; Li *et al.* 2007; Zhao *et al.* 2009), the use of sound for perception (Griffin 1958) and a wide range of ecological topics (Kunz 1988). Bats also have important roles in temperate and tropical ecosystems. In temperate climates, the majority of bats are insectivores, consuming huge amounts of prey (up to half of their own weight every feeding night; Federico *et al.* 2008; Kalka *et al.* 2008; Williams-Guillén *et al.* 2008). In addition, in tropical climates bats also have an important role in pollinating and dispersing seeds of many species plants, being especially relevant in the recovery of tropical forests (Whittaker & Jones 1994; Kelm *et al.* 2008).

Over the 20th century, occurred a general decline in bat populations throughout Europe, with roost disturbance/destruction, use of pesticides and destruction of foraging habitats the most common and serious threats (Stebbings 1988; Hutson *et al.* 2001). More recently, collisions with wind turbines and road kills have also increased pressures on bat populations (Kunz *et al.* 2007; Arnett *et al.* 2008; Rodrigues *et al.* 2008; Kerth & Melber 2009). Bats are especially vulnerable to extinction due to their slow population growth, late sexual maturity and their frequent dependence on specific habitats for foraging and roosting (Jones *et al.* 2003; Kafi & Kerth 2004). This implies that when a population suffers considerable losses it will take a long time to recover to its initial condition (Bell & Collins 2008). If threats persist, then extinction risk is increased.

Globally, the structure and function of ecosystems are changing at an unprecedented rate, primarily because of human activities associated with provision of food and fibre, and because of the emission of greenhouse gases and the use of carbon-based resources for energy (Vitousek *et al.* 1997; Tilman *et al.* 2001). The impact of two distinctive types of global change must be monitored to better understand the conservation threats to bats. The first is the alteration of conditions associated with climate change (e.g. regional alterations in temperature and precipitation; Walther *et al.* 2002; Parmesan & Yohe 2003), and the second is the conversion of natural habitats to those used for the support of human populations (e.g. forestry, agriculture, industry and urbanisation; Vitousek *et al.* 1997; Jones *et al.* 2009). In this thesis I will address both the potential impacts of changes in climate and habitat on threatened bats in Europe.

1.3 Applications of molecular analysis in bat conservation

The use of molecular data for conservation purposes is assuming a greater relevance. Understanding the population structure of a species can frequently be used to infer both past and current behavioural processes and hence population history (Burland *et al.* 1999). The identification of isolated populations or unique gene pools through genetic analysis (e.g. Dallas *et* al. 1995, Burland *et al.* 1999) has been important to identify areas that require special protection – management units (e.g. Salgueiro *et al.* 2003; Michaux *et al.* 2004). The advent of molecular genetic techniques has also provided an opportunity to address questions of the nature of population structure in bats more fully (Burland *et al.* 1999). The ability to fly gives bats an excellent capability to disperse. However, many factors including migratory and mating behaviour, physical barriers to gene flow and historical colonisation patterns can influence the extent to which population boundaries are genetically defined (Burland & Worthington Wilmer 2001). At an inter-population level, molecular studies have shown a great genetic diversity within bats (Burland & Worthington Wilmer 2001). Even so, for the majority of temperate bats females show a marked philopatry, with social structure having a strong influence on a species' population structure (Kerth *et al.* 2008). For example, Burland *et al.* (1999) found genetic isolation among maternity colonies of *Plecotus auritus* that were located in close proximity to each other and had no physical barriers between them. These authors also expected that this pattern would appear in other bat species with similar wing morphology and flight capability (e.g. Kerth *et al.* 2002; Chen *et al.* 2008). Furthermore, the rarity and small population size

of some species may also contribute to their isolation. Isolation typically depletes genetic variation, for example by reducing gene flow, and by increasing genetic drift and inbreeding (Hedrick & Kalinowski 2000; Frankham 2005; Durrant *et al.* 2009). On the other hand, outbreeding promotes survival in at least one well-studied bat species (Rossiter *et al.* 2001).

Additionally, in recent years other applications of genetic analysis in the study of bats has allowed the discovery of several cryptic species in Europe, such as *Pipistrellus pygmaeus* (Jones G. & van Parijs 1993; Barratt *et al.* 1997), *Myotis alcathoe* (von Helversen *et al.* 2001) and several other species especially in southern Europe (Mayer & von Helversen 2001; Ibáñez *et al.* 2006; Spitzenberger *et al.* 2006). As a consequence, the number of bat species in Europe has increased from around 30 recognized species in the early 1990s to ca. 40 nowadays (Dietz & von Helversen 2004).

Furthermore, the combined use of molecular biology with other research areas such as ecology, behaviour and bioacoustics has also allowed the development of more adequate management recommendations for several species (Newton *et al.* 2003; Rivers *et al.* 2005; Davidson-Watts *et al.* 2006). The development of integrative studies is currently a field in expansion, where several approaches are still being assayed and new concepts arise frequently (e.g. Manel *et al.* 2003; Kidd & Ritchie 2006).

1.4 Species distributions and knowledge gaps

Geographic Information Systems (GIS) are best known as a tool for the creation and editing of maps (Johnston 1998). However, in recent years the capability of GIS has grown so much, that it is now possible to integrate

different levels of information and generate predictive distribution models for species and foraging habitats (e.g. Jaberg & Guisan 2001; Wang *et al.* 2003). In fact, any variable that has a spatial component can be submitted to analysis in a GIS. When used together with geostatistics, multivariate statistics and landscape modelling it is possible to detect and predict complex ecological patterns (Johnson & Cage 1997).

In-depth knowledge of a species' distribution is of prime interest for conservation management (Jaberg & Guisan 2001). Modern conservation ecology crucially relies on multivariate, spatially explicit models that describe and/or predict a species' distribution (Hirzel *et al.* 2002).

By combining species distribution modelling with other areas of interest, like molecular biology and morphometrics (Kidd & Ritchie 2006; Brito *et al.* 2008), it is now possible to combine and project data in order to obtain spatially meaningful results. This approach has already been used in a range of phylogeographic (e.g. Hugall *et al.* 2002; Kidd & Ritchie 2006; Dépraz *et al.* 2009), evolutionary (e.g. Ritchie *et al.* 2001) and conservation studies (e.g. Sattler *et al.* 2007). Nonetheless, the use of species distribution modelling for rare species and, specifically for bats, has seldom been assayed (Tsoar *et al* 2007). The rarity of some species challenges model performance severely, because datasets are probably composed of a low number of occurrences and include geographical sampling biases, resulting either from surveying only a fraction of the area where a species occurs, or a fraction of ecological conditions (e.g. habitat, climate) where the species is able to subsist (Hortal *et al.* 2008). The consequences of these constraints and biases on model performance are still very much unknown (Jiménez-Valverde *et al.* 2008).

1.5 Purpose of this research

To respond to the current biodiversity crisis, and specifically to the suspected decline of populations of several European bat species, conservation biology needs to integrate novel techniques in ecology as they emerge. New and more detailed information on a species' status, its distribution, population structure and ecological needs is paramount for an efficient implementation of management policies and determination of causes of threat. Yet, this should not be done blindfolded. As in all fields of science, the application of modern techniques should be tested, giving a major focus on rare species. This study aims to combine genetic analysis with predictive modelling over a range of spatial and temporal scales. By choosing a rare bat as the study species, model performance is tested with a dataset that possesses several inherent constraints (small sample size and sampling bias), a situation similar to that experienced by several other rare species. Yet, possible outcomes are of extreme interest for conservation management after proper validation.

The focal bat species for this thesis was chosen on the basis of a lack of knowledge and a high level of threat. Therefore, this work mainly focuses on the barbastelle, *Barbastella barbastellus* (Schreber 1774), a tree-dwelling species and one of the rarest bats in Europe (Urbańczyk 1999). Barbastelles seem to be dependent upon mature broadleaved woodlands for roosting and foraging (Russo *et al.* 2004; Hillen *et al.* 2009), and this dependence (together with the disappearance of its habitat) is the probable cause of its suspected population decline. This species has the status of "Data Deficient" in the

Portuguese Red Data Book (Cabral *et al.* 2005) and it is considered "Vulnerable" at a global scale by the International Union for Conservation of Nature (IUCN; Vié *et al.* 2009).

The work presented in this thesis used two different geographical scales: Portugal (country scale) and Europe also including parts of north Africa (continental scale). Fieldwork was carried out in mainland Portugal covering ca. 90 000 km^2. Despite its small area, Portugal possesses high levels of biodiversity, and indeed is one of the European countries with the highest number of threatened species (Vié *et al.* 2009). Regarding bats, 24 species are acknowledge to exist in mainland Portugal, of which nine have a recognised threat status (three Critically Endangered, one Endangered and five Vulnerable) and other nine have insufficient data for evaluation in the Portuguese Red Data Book (Cabral *et al.* 2005). The country is located in the Iberian Peninsula. Strong climatic and biogeographic influences from both Africa and Europe have created a diverse mosaic of habitats and conditions that contribute to the number of species present, hence validating its inclusion in one of the world's biodiversity hotspots - the Mediterranean basin (Schipper *et al.* 2008). From the legal point of view, the country undersigns the majority of Environmental International agreements with ca. 21% of the land classified as protected areas. Moreover, Portugal also had the first developed and approved bat conservation plan in Europe (Palmeirim & Rodrigues 1992). Nevertheless, the implementation of environmental policies has been very weak, hence major threats to biodiversity still persist (Moreira & Russo 2007; Ribeiro & Lovett 2009).

Additionally, this thesis also uses modelling integrated with genetic analysis at a continental scale – covering Europe and part of north Africa. Therefore, analyses over this extensive area covered much of the geographical range of European near-endemic bat species, as for example *B. barbastellus.* Due to the wide area involved, all fieldwork was carried out only in Portugal. Samples for genetic analysis were provided by several collaborators, while data for modelling was obtained from several European databases available online.

Finally, this work also focuses on the potential impact of climate change on European bats. Because the climate change study did not require tissue samples or individual bat measurements, a variety of databases could be analysed. It was possible to extend the investigation on climate change impacts to several other bat species.

The proposed objectives of this thesis are:

1 – To investigate and test novel species distribution modelling techniques and their performance by using *B. barbastellus* as a model species.

2 – To combine species distribution modelling techniques with genetic analysis to fill knowledge gaps and allow the development of the first conservation measures for *B. barbastellus* in Portugal.

3 – To investigate the phylogeography of *B. barbastellus* in Europe by integrating paleo-distribution modelling with phylogenetic analysis of mitochondrial DNA (mtDNA) sequences.

4 – To study the potential impact of climate change on European bat diversity with respect to the biogeographic affinities of taxa.

1.6 Thesis outline

The structure of this thesis has a step-by-step approach, whereby the specific problem investigated in each chapter is addressed and allows progression to another problem that is analysed in the subsequent chapter. The initial chapters try to validate species distribution modelling and its integration with genetic analysis on a rare species at a country scale - Portugal. With information gathered on methodological constraints, the last chapters widen the scope of the applicability of the modelling approaches, by applying them to past conditions and to future climate change scenarios at a continental scale.

Chapter two investigates the performance of two presence-only modelling techniques by ground-truthing the predicted distribution of *B. barbastellus* in Portugal. Results show that the Maximum Entropy (Maxent) technique outperformed Ecological Niche Factor Analysis, allowing the discovery of new populations and extending the known range of the species considerably.

Having obtained support for the validation of Maxent in the previous chapter, in chapter three the predicted distribution of the bat in Portugal is updated with newly gathered occurrence data, and results from respective models are combined with analysis of population structure obtained by analysis of mtDNA sequences. Consequently, the first management measures are suggested for this species in Portugal, taking into account its population structure, predicted distribution and associated ecological constraints.

In chapter four the main aim is to investigate the impact of the Last Glacial Maximum (LGM) and postglacial events on the distribution and population structure of *B. barbastellus* across its entire range (excluding the Canary Islands). By integrating species distribution modelling for the current situation and LGM distributions with genetic phylogeographic inferences, this study aims to provide a better understanding of the location of glacial refugial populations and postglacial colonisation routes. These results also allowed investigation of whether climatic niche conservatism occurred over long time periods in *B. barbastellus* populations. The agreement of results obtained by predictive modelling and through molecular analysis supported the projection of models developed from current conditions to other sets of climatic conditions, like future scenarios of climate change. Finally, a significant conservation output was also achieved through the definition of European management units for the species.

Chapter five has a broader application, with 28 bat species selected for study. By using predictive modelling to investigate the potential impact of climate change in the 21st century, it is predicted that European bat diversity may suffer severe challenges in the future. Moreover, because bats were grouped in the analysis according to their biogeographic affinities (and not considered as a single unit), it was possible to detect problems in biogeographic groups that have a lesser number of species within this taxon.

CHAPTER 2

Ground validation of presence-only modeling on rare species: a case study on barbastelles *Barbastella barbastellus* (Chiroptera: Vespertilionidae)

Abstract

1. This study evaluates the accuracy of presence-only modelling for predicting the distribution of rare species, when data are scarce and usually collected with sampling biases. I modelled the potential distribution in Portugal of one of the rarest European bats, *Barbastella barbastellus* and subsequently ground-validated model predictions by using acoustic transects.

2. I used Ecological Niche Factor Analysis (ENFA) and Maximum entropy modelling (Maxent) to build habitat distribution models for *B. barbastellus*, and determined which ecological factors were most relevant for each model. Because ENFA only accepts continuous variables, one Maxent model was built using the same variables as ENFA and another using land cover as a categorical variable.

3. ENFA and both Maxent models predicted similar areas of occurrence in central and northern regions of Portugal, although ENFA predicted suitable habitat over a wider range. Conversely, there was substantial disagreement on the location of high-suitability areas in the south. This could be a consequence of a different choice of important variables made by each model. Native woodland and average temperature were the most relevant variables for Maxent, while in ENFA *B. barbastellus* was linked to higher altitudes though it was predicted to avoid production forests and infrastructures.

4. Threshold-independent and dependent statistics showed that Maxent models outperformed ENFA, probably as a consequence of divergent predictions in the extrapolation area. Overall, 15 new *B. barbastellus* sites were discovered (at least 30 km from previous records) and known distribution was extended approximately 100 km to the south.

5. *Synthesis and applications:* This study results support the use of presence-only modelling as an indispensible tool for survey design as shown by the discovery of *B. barbastellus* populations outside of the previously known range. ENFA seems to be more suited to determine a species' potential distribution, although failing to extrapolate it. In contrast, Maxent is better suited to determine a species' realized distribution. Its successful predictions in the extrapolation area recommend this technique for determination of a conservative distribution for a species. Maxent modelling would greatly aid biodiversity conservation, especially when it is necessary to develop survey plans or the first assessments of a species' distribution.

2.1 Introduction

An accurate knowledge of a species' distribution is of prime interest for conservation management (Jaberg & Guisan 2001). Over the last two decades, several multivariate techniques have been developed to predict species' distributions, such as logistic regressions (Brito *et al.* 1999), discriminant analysis (Manel *et al.* 1999), artificial neural networks (Manel *et al.* 1999; Spitz & Lek 1999), Generalized Linear Models and General Additive Models (Brotons *et al.* 2004), Bioclim (Tsoar *et al.* 2007) and Genetic Algorithm for Rule-set Prediction (GARP; Stockwell & Peterson 2002). All these methods try to establish a link between the focal species and spatial variables such as topographical features (e.g. altitude, slope), ecological data (e.g. frequency of forests, mean temperature) or infrastructures (e.g. road density, distance to a reservoir). For these methods, the data regarding the focal species are usually described in the form of presence/absence in a set of sampled locations. However, absences could include "false absences", hence biasing the model's predictions. False absences arise in situations where the species could not be detected even though it was present (Hirzel & Le Lay 2008). False absences are especially relevant when studying bat species, because intensive population surveys are difficult to conduct. Bats have a nocturnal and elusive behaviour with large home ranges and problems can exist with the identification of species in flight (Walsh & Harris 1996).

Presence-only modelling appears to be an alternative technique to address these constraints because it does not require the existence of absence in the data. In recent years several presence-only techniques have been developed, and their reliability has been tested for different sample sizes and spatial scales

focusing most frequently on models such as GARP, Domain, Bioclim and Maxent (Stockwell & Peterson 2002; Elith *et al.* 2006; Hernandez *et al.* 2006; Pearson *et al.* 2007; Tsoar *et al.* 2007; Wisz *et al.* 2008). These techniques are assuming a greater relevance for species conservation, especially for rare or poorly documented species, with some showing strong performance even with scarce presence data (Papeş & Gaubert 2007). Moreover, some of these techniques seem to be robust to non-systematic data (although a model's best performance is achieved from systematic surveys) which is especially relevant when working with the available presence data collected by different researchers and methodologies, e.g. museum collections or herbaria (Hirzel *et al.* 2002). In fact, datasets are typically characterized by sampling biases where available data usually do not cover the whole range of ecological conditions in which the studied species may occur (Reddy & Dávalos 2003; Wisz *et al.* 2008). The effect of sampling bias can have a severe impact on model quality, nevertheless it has received little attention in the predictive modelling literature (Randin *et al.* 2006; Peterson *et al.* 2007; Hortal *et al.* 2008; Phillips *et al.* 2009). As such, it has been stressed that more research should focus on the effect of sampling biases on model predictions (Pearson *et al.* 2007; Elith & Graham 2009; Phillips *et al.* 2009). In this study, two presence-only modelling techniques were compared, Ecological Niche Factor Analysis (ENFA) and Maximum Entropy (Maxent), regarding their efficacy in predicting new areas of occurrence for a rare species. These techniques differ in their modelling approaches with Maxent theoretically more suited to predict the realized distribution of a species, defined as the locations and range of environmental conditions in which a species actually lives (Elith *et al.* 2006; Jiménez-Valverde

et al. 2008). ENFA generally provides predictions closer to the potential distribution or the distribution a species could inhabit if it occupied all areas with favourable conditions (Hirzel & Le Lay 2008). To my knowledge these techniques have never been compared despite becoming widely used.

There are several statistical methods to validate habitat suitability models including Receiver Operating Characteristics plots (ROC), the Boyce index, Kappa statistics, and null models (Fielding & Bell 1997; Liu *et al.* 2005; Hirzel *et al.* 2006). However, the majority of the modelling validation methods involve comparing subsets of the presence data with the predictions generated by the models. Very often the models give new predicted areas of occurrence for the studied species, and these are clearly among the most relevant outputs of ecological modelling (Hernandez *et al.* 2006). However, new areas of predicted occurrence are rarely verified in the field (Greaves *et al.* 2006) which is of concern, especially for areas outside the known geographical range of the species (Elith *et al.* 2006; Randin *et al.* 2006). Such a lack of validation is highly relevant for rare species, which are usually a conservation priority, and for which the knowledge gap is generally wider than for more common species. I chose to study one of the rarest European bat species, the barbastelle *Barbastella barbastellus* Schreber, 1774. This tree-dwelling bat is widespread in Europe although its distribution is highly fragmented (Mitchell-Jones *et al.* 1999). It seems to be dependent on native mature woodland, so deforestation and habitat fragmentation are probably associated with its suspected population decline (Russo *et al.* 2004). In this study, I modelled and tested *B. barbastellus* distribution in Portugal, where the species was first discovered in 1980 (Palmeirim 1990), and for which only a few locations were known by

2004. Two major bioclimatic zones exist in the country, the Atlantic and the Mediterranean (Sillero *et al.* 2009). Almost all of known *B. barbastellus* locations were situated in the Atlantic bioclimatic area in the centre and north of the country, hence presenting a clear example of presence data that probably included sampling biases. By modelling this bat distribution in Portugal it would be also possible to evaluate the accuracy of model projections in different bioclimatic zones and hence to test their behaviour under a sampling bias in the presence data. I believe that this study mirrors the limitations of working with rare species in general: data are likely to have sampling biases, while model extrapolation to new areas outside the known range is one of the most important and desirable outcomes expected from distributional modelling (Phillips 2008). As such the main objectives of this study were: (1) to investigate both modelling techniques regarding their predictions and discrepancies for the distribution of *B. barbastellus* in Portugal; (2) to determine which ecological factors are relevant for each model; and (3) to validate these modelling techniques by comparing predicted distribution with results obtained by acoustic transects.

2.2 Methods

2.2.1 Study area

The study was carried out in mainland Portugal (approximately between coordinates 36°N - 41°N and 7°W - 9°W). Two major climate zones divide the country. In the north and in the majority of the central parts of the country the Atlantic temperate climate dominates with mild summers and cold, rainy winters. The landscape is mountainous with native forests mainly composed of

oaks (*Quercus robur*, *Q. pyrenaica*, *Q. faginea*), chestnut (*Castanea sativa*), birch (*Betula* spp.), ash (*Fraxinus* spp.), and other flora that constitute a typical European Atlantic forest (Fig. 2.1). The southern regions are characterized by a Mediterranean climate with mild winters and hot, dry summers. As a consequence, the landscape is dominated by an oak savannah-like woodland (*Quercus suber* and *Q. ilex*) spreading over vast plains (Fig. 2.1) (Sillero *et al.* 2009).

2.2.2 Presence data and environmental variables

For model calculation I used all known locations of *B. barbastellus* in Portugal (N=17) since its discovery in 1980 up to 2004 (source Instituto da Conservação da Natureza e Biodiversidade; Fig. 2.2) as the dependent variable. Only records with a finer resolution than 30 arc seconds were considered for modelling. Moreover, a set of independent ecogeographical variables (EGV) was selected as environmental predictors: annual average temperature (ºC), annual average precipitation (mm), annual average temperature range (ºC), altitude (source WorldClim; http://www.worldclim.org) and land cover (source Global Land Cover 2000; http://www-gvm.jrc.it/glc2000/ and Instituto Geográfico Português). This latter EGV was reclassified into six classes namely, infrastructures (e.g. towns and villages, industrial areas, roads), agriculture fields including steppes, production forests (mainly conifer and *Eucalyptus* spp.), scrubs and regenerating forest, native woodland and water bodies. Although, land cover data refer to recent years while presence data come from as early as 1980, this was not relevant for modelling because areas

Figure 2.1 – Photographs representative of the Atlantic forest in the north and centre of Portugal (left photo) and the oak savannah-like woodland in the south (right photo).

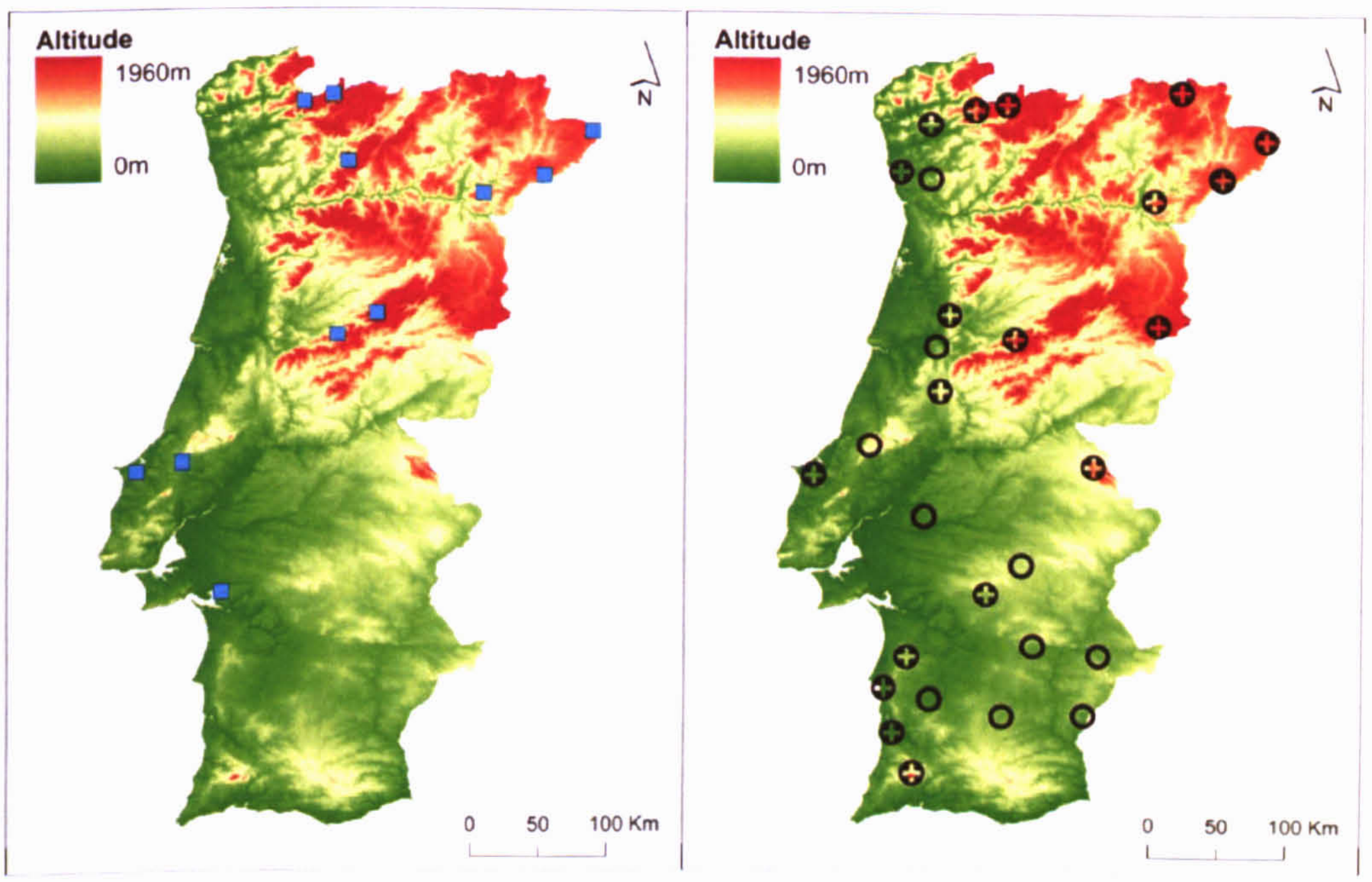

Figure 2.2 – Maps with the location of the training (left) and test (right) data. Squares on the training data map indicate the location of the occurrence records used to build the models. Each square may contain more than one location. The test map shows the location of the acoustic transects where empty circles represent absences (or unconfirmed presences) while circles with crosses within represent confirmed presences. Each symbol may contain more than one transect.

where *B. barbastellus* was found remained fairly constant over time, i.e., those areas of mature woodland still remain intact. Regarding chosen climatic variables, I was limited by the low number of presences in the training data. Thus, variables chosen are known to have a great relevance on bat physiology and survival: temperature and water availability (Baken & Kunz 1988), here expressed as two temperature variables and by precipitation. All digital information had a resolution of 30 arc seconds (~900 m), thus the study area included 133 291 cells corresponding to an area of approximately 90 100 km^2.

2.2.3 Modelling procedure

Two recently developed presence-only modelling techniques were used to build habitat suitability maps for *B. barbastellus* in Portugal, which involve different theoretical approaches (see this chapter introduction) and which have not been tested through ground-validation: Ecological Niche Factor Analysis (ENFA) and Maximum Entropy (Maxent).

For computing ENFA models, Biomapper v3.1 to v.4 were used (Hirzel *et al.* 2007; http://www2.unil.ch/biomapper/). Because all land cover data were in a Boolean format, quantitative variables were derived from the original ones since this is a requirement for ENFA. Therefore, the frequency within a 2.5 km radius (proportion of cells with the attribute within that radius) was calculated for the following EGVs: agriculture, production forest, scrub and regenerating forest, native woodland and water bodies. I also calculated the distance between the focal cell and the cells where infrastructures were present. The CircAn and DistAn modules in the Biomapper software package were used to perform these operations (Hirzel *et al.* 2007). Altitude, precipitation and temperature

variables were obtained directly as quantitative data. Prior to the development of the models, all EGVs were normalized using the Box-Cox transformation (Sokal & Rohlf 1995) as available in the Biomapper software.

ENFA then summarizes all ecological variables into a few uncorrelated factors in a similar way to principal component analysis, and compares the species distribution with all employed EGVs. The number of factors to be included in the model was obtained by comparing each factor's eigenvalue with the MacArthur's broken stick distribution (Hirzel *et al.* 2002). All computation behind this model is based on the niche concepts of marginality and specialization. For this analysis a niche is essentially the subset of EGV values where the species occurs (Hirzel & Le Lay 2008), thus the method seems to be appropriate for estimating a species' potential niche. Marginality measures how the average conditions over a species' distribution differ from the average conditions in the study area. Specialization is defined as the species' niche breadth, or how wide the range of EGV values is over the area in which the species is found. In this study, instead of specialization I present the score for tolerance (the inverse of specialization), which is easier to interpret because its values range between 0 and 1 (where values closer to 0 indicate higher specialization). The habitat suitability map was then computed using harmonic means because this algorithm seems to be more adequate for data from rare species (Hirzel & Arlettaz 2003). The analysis produces habitat-suitability maps (whose pixel score varies between 0 and 100) for the focal species and subsequently allows the determination of the most important ecological predictors associated with the presence of a species.

Maxent is a machine-learning process employing a statistical mechanics approach that also uses presence-only data. Maxent estimates the range of a species by finding the maximum entropy distribution (i.e. closest to the uniform) given the constraint that the expected value for each EGV closely matches the empirical average of the occurrence data (Phillips *et al.* 2006). It differs from ENFA by this approach which makes it more suited to describe a species' realized niche. Maxent then weights each EGV by a constant and the modelled probability is a Gibbs distribution (i.e. exponential in the sum of the weighed EGVs), divided by a scaling constant to guarantee that the probability ranges from 0 to 1 (Phillips & Dudík 2008). Gain (a measure of the likelihood of the samples with the EGVs) represents how much better the distribution fits the sample points than the uniform distribution does (Phillips *et al.* 2006). To check which variables were the most important for model building, a Jackknife analysis of the gain was made with the presence data. During this process, a number of models are generated. First, each EGV is excluded in turn and a model created with the remaining variables in order to check which variable is the most informative. Then, a model is created for each individual EGV to find which variable has the most information that is not present in the others, i.e., the most uncorrelated variable. In order to test if the normalization of the EGVs had impact on Maxent's performance I calculated two models. One used exactly the same continuous variables as the ones employed in ENFA (hereafter this model will be called Max_cont). Because categorical variables can be used in this algorithm, another model was calculated using land cover in a binomial format (hereafter this model is called Max_cat). All calculations

were done in Maximum Entropy Species Distribution Modelling v2.0 and v3.2.1 (http://www.cs.princeton.edu/~schapire/maxent/).

2.2.4 Acoustic transects and sound analysis

In order to ground validate the habitat suitability models, acoustic surveys were conducted consisting of a 30 minute transect walked at a regular pace using an ultra-sound detector (D-240x Pettersson Elektronik AB), so that around 1 km was covered (although transect length may varied according toterrain characteristics). Transect surveys started at civil twilight after sunset and lasted for the following 2 h 30 mins in order to match the peak activity period of *B. barbastellus* (Russo *et al.* 2007). To avoid auto-correlation between transects on the same night, surveys were done at least 5 km from each other. The average *B. barbastellus* home range has been reported to be around 5 km in the U.K. (Greenaway 2001; Davidson-Watts, pers. comm.), hence with this distance between transects it was highly unlikely that the same individual would be recorded in different areas. Whenever weather conditions were adverse for bat activity, such as strong winds or rain, transects were cancelled (Russo & Jones 2003; Rainho 2007).

Surveys were done with the heterodyne output of the detector tuned between 32-34 kHz to match the frequencies of maximum energy emitted by *B. barbastellus* (Denzinger *et al.* 2001; Russo & Jones 2002). All detected bat calls were then recorded in time-expansion mode onto a digital recorder (Edirol R-09; files saved in WAV format; sampling rate 44.1 kHz and 16 bits/sample) and analysed with sound-analysis software (BatSound Pro v3.31b, Pettersson Elektronik AB) using a 512 pt FFT with Hamming window for spectrogram

analysis. Call parameters were measured using crosshair screen cursors; temporal variables were measured from oscillograms, while frequencies were taken from power spectra. *B. barbastellus* has distinctive echolocation calls that allow reliable acoustic identification characterized by bi-tonal vocalizations (which is unique among European bats), frequency of maximum energy between 32-34 kHz and interval between pulses ca. 100 ms (Denziger *et al.* 2001; Russo & Jones 2002). Transect results were used later as test data to evaluate model performance. This is a crucial aspect of this work because test data were in a presence/absence format obtained independently of the training data, rather than consisting of a random background or a subset of the initial dataset as is frequently used (e.g. Elith *et al.* 2006; Martinez-Freiría *et al.* 2008). Ground validation data were obtained with a better or equal resolution than model's training data (30 arc seconds).

Transect locations were defined to cover the range of model scores proportional to the amount each cell values occupied area in the study area (Fig. 2.3). This way, the more frequent cell values were more sampled than the less frequent ones. Additionally, transects were planned to cover the extent of the whole study area (Fig. 2.2). Whenever possible, we repeated transects where *B. barbastellus* presence was not confirmed, taking care never to replicate them in the same season of the year. *B. barbastellus* is often associated with mature woodland close to water sources (Sierro 1999; Greenaway 2001). Thus, transects were located in habitats as similar as possible to those containing these features. Between the months of April-September of 2005-2007 72 transects were walked in 52 different locations, and 121 *B. barbastellus* passes were recorded (average of 1.7 ± 3.3

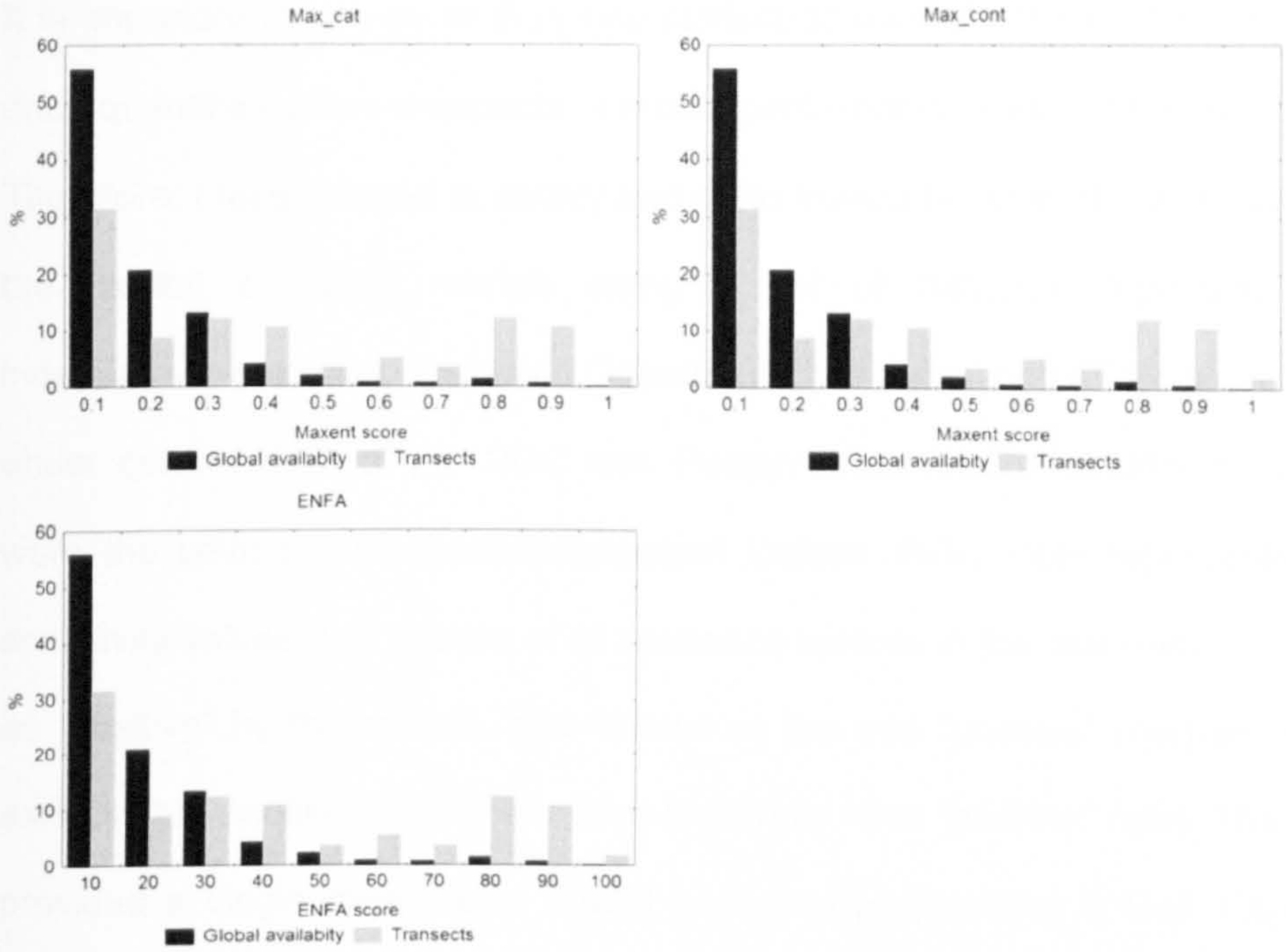

Figure 2.3 – Proportion of each model cell values in the study area (global availability) and in the location of the acoustic transects. See model definitions in Methods.

passes/transect) of 1530 analysed recordings belonging to other 15 bat species. I acknowledge that a higher number of transects would increase the power of the statistical tests, however this was limited by the extensive logistics involved.

2.2.5 Model evaluation

I started by calculating a two-tailed Wilcoxon signed rank test using the cell values to test if the models generated different predictions (Randin *et al.* 2006; Phillips *et al.* 2009). Additionally, I calculated pairwise linear regressions of the models using cell values where *B. barbastellus* presence was confirmed, which also allowed investigating if the transformation of categorical to continuous variables had any influence on model predictions.

It is important to use more than one statistic to evaluate the models because each quantifies different aspects of model performance (Elith & Graham 2009). Therefore, I tested model accuracy and performance between the test data and the habitat suitability models using a set of threshold-dependent and independent statistics. Receiver Operating Characteristics (ROC) plots, area under curve (AUC) of the ROC and Pearson's correlation coefficient (COR) were the selected threshold-independent indices. ROC plots represented all sensitivity values (the fraction of all presence records in the test data classified as "positive" by the models, also known as the true "positive" rate) on the *y*-axis, plotted against 1-specificity (also known as false "positive" rate). The AUC provides a single measure of model performance (Fielding & Bell 1997) by giving the probability that the model correctly ranks a random presence site vs. a random absent site (Phillips *et al.* 2009). It ranges from 0.5 (randomness) to 1 (perfect discrimination). The correlation, COR, between the test data and model predictions is known as the point biserial correlation and can be calculated as a Pearson correlation coefficient (Elith *et al.* 2006). It differs from AUC by measuring the degree to which the predictions vary with the acoustic transect data, thus it is likely to be more sensitive to the relative sampling intensity (Phillips *et al.* 2009).

The model performance after model reclassification into binary maps (or presence/absence) was also tested, since this is one of the most important and widely used outcomes for applications such as biodiversity assessment, reserve selection and climate change predictions (Lobo *et al.* 2008). The definition of the threshold should not be chosen arbitrarily but based on the objectives of the modelling (Hernandez *et al.* 2006) taking into account the

quality and precision of the data employed. However, there has been a lack of agreement on the criteria that can be used to define a threshold in presence-only modelling. The 10th percentile presence value (above which it is considered that the species is present) assumes that 10% of presence data may suffer from errors or lack of resolution (Raes *et al.* 2009). This is especially relevant when dealing with data sets gathered over large time-spans where reliability and precision has probably varied. Thus, this threshold value was used to reclassify these models and subsequently for calculation of the threshold-dependent statistics, namely Cohen's Kappa, correct classification rate, sensitivity and specificity. The Kappa statistics (Cohen 1960) define the accuracy of the prediction and range from -1 to +1, where +1 indicates a perfect agreement between test data and predictions, while 0 indicates an agreement no better than random. The correct classification rate indicates the proportion of correctly classified presences and absences of the test data according to the defined threshold (Barbosa *et al.* 2009). Additionally, by calculating sensitivity and specificity I evaluate how well models identify presences and absences, respectively (Lobo *et al.* 2008; Jiménez-Valverde *et al.* 2008). All statistical analyses were performed using SPSS v15.0 (Illinois, Chicago).

2.3 Results

2.3.1 Analysis of the model predictions

The three models predicted the majority of the high suitability areas in the central and northern regions of Portugal (Fig. 2.4), although they differed in predicting the extent of that area. The area of high suitability under Max_cat

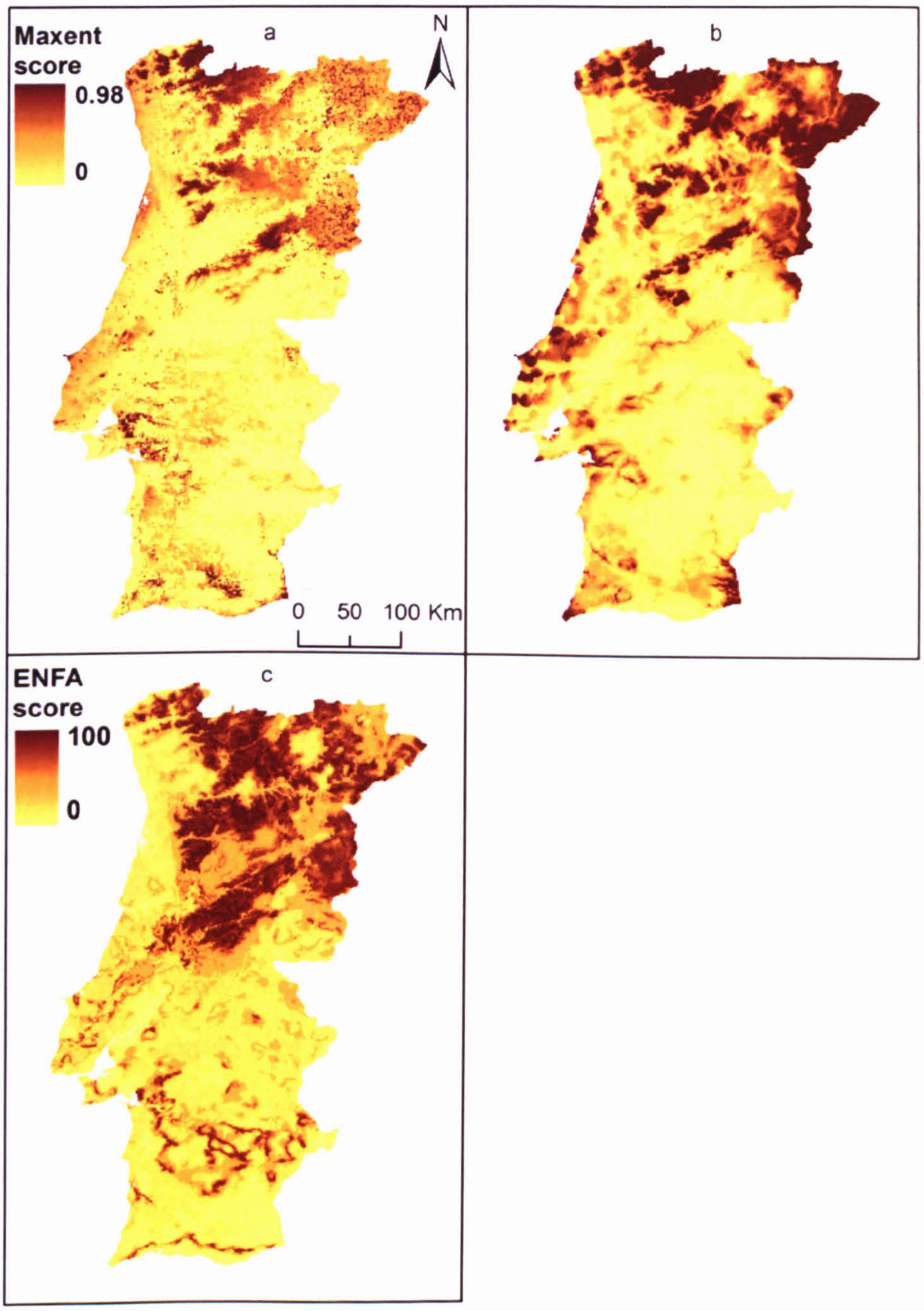

Figure 2.4 - Habitat-suitability maps for *Barbastella barbastellus* in Portugal, as computed by the three models: (a) Maxent including categorical variables, (b) Maxent with continuous variables only and (c) ENFA.

was the most fragmented of all models, whereas in ENFA suitable areas showed a higher potential for population connectivity over a wide region (Fig. 2.5).

On the other hand, predictions of suitable area in the south clearly differed between both Maxent models and ENFA. The highest Maxent scores were located in mountainous areas or close to the coastline, while in ENFA equivalent areas were mainly located in the steppe and agricultural plains.

2.3.2 Determination of most relevant ecogeographical variables for each model

ENFA provided an overall marginality of M=1 and an overall tolerance T=0.27, meaning that *B. barbastellus* lives in extreme habitats compared with what is available on average in Portugal, and also that the species has a narrow niche breadth, i.e., it is somewhat restricted by the range of conditions it can withstand.

The first four factors were selected as significant (Table 2.1), explaining 100% of the marginality (in ENFA the first axis always accounts for 100% of marginality) and 93% of specialization. The marginality factor alone explained 24% of the specialization, showing again that this species occupies a very restricted range of conditions that differ from average conditions found in Portugal.

The ENFA results indicated that the presence of *B. barbastellus* was linked to lower temperature ranges than those found on average in Portugal and to higher altitudes and that the bat avoided areas of infrastructure, agricultural fields and production forests, preferring dense areas of native woodland.

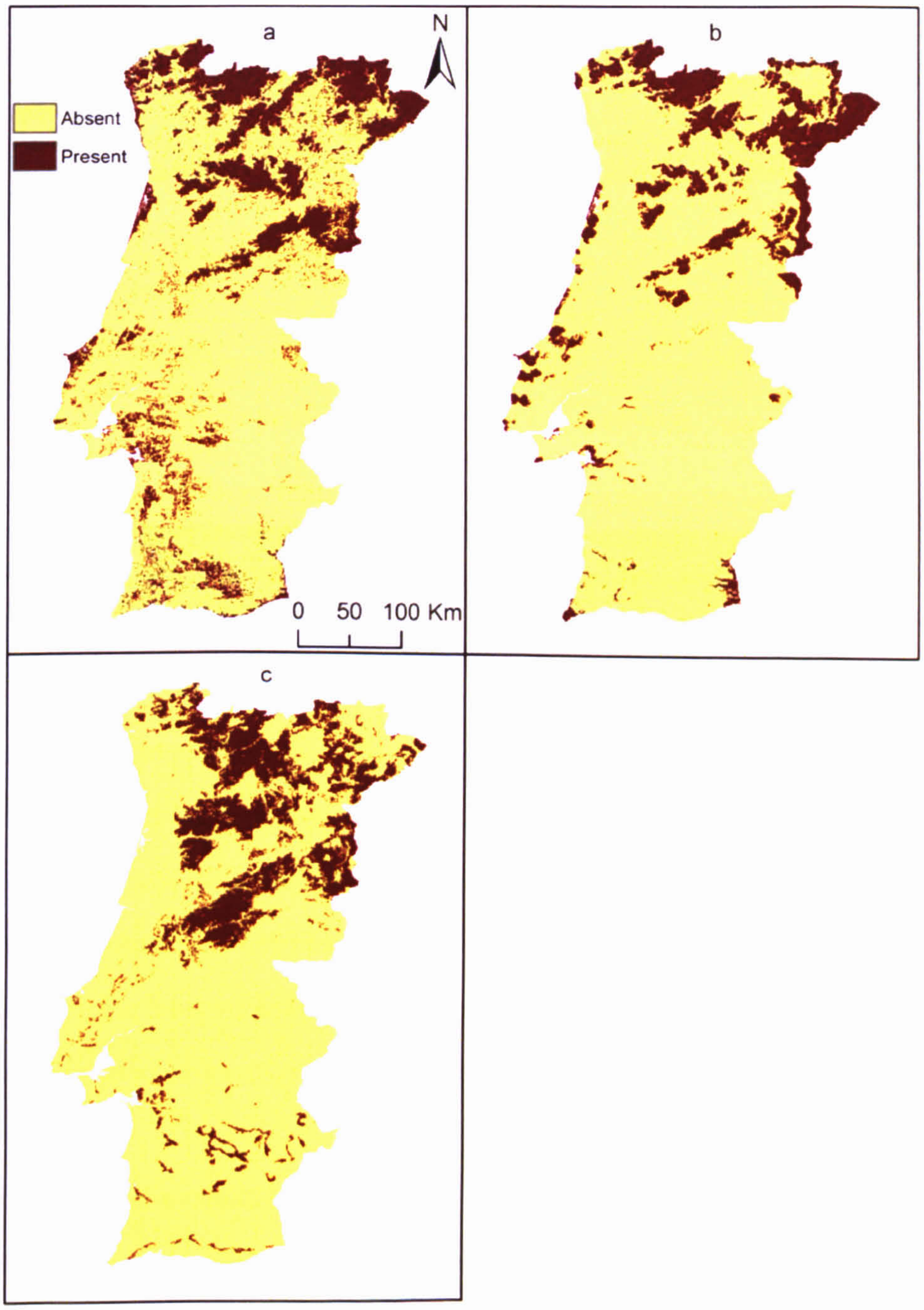

Figure 2.5 – Reclassified habitat suitability models as computed by the three models: (a) Maxent including categorized variables, (b) Maxent with continuous variables only and (c) ENFA. Threshold definition for the production of the binary maps may be found in the Methods section.

	Marginality	Specialization		
EGV	Factor 1 (24%)	Factor 2 (34%)	Factor 3 (22%)	Factor 4 (13%)
Agriculture	-0.217	-0.309	**0.500**	**0.404**
Altitude	**0.439**	**0.497**	0.210	-0.331
Native woodland	0.085	0.051	**0.566**	0.056
Human infrastructures	**0.423**	-0.189	0.109	-0.109
Precipitation	0.365	**-0.548**	0.221	0.085
Production forest	-0.201	**0.436**	**0.564**	**0.766**
Scrubs & regenerating forest	0.194	0.255	0.176	0.262
Annual temperature	-0.035	-0.035	-0.004	0.202
Water bodies	0.202	0.012	-0.003	0.047
Temperature range	**-0.564**	0.251	0.402	-0.082

Table 2.1 - Correlations for the first four factors of Ecological Niche Factor Analysis (ENFA) for *Barbastella barbastellus* records in Portugal. Percentages indicate the amount of variance explained by each factor. Marginality factor: values higher than 0 indicate a higher correlation between the bat presence and the ecogeographical variable (EGV); positive and negative signs indicate selection or avoidance of the EGV, respectively. Specialization factor: higher values indicate a higher specialization for the EGV; positive and negative signs have no meaning for this factor. Values higher than 0.4 are in bold for an easier visualisation of the table.

Maxent jackknife analysis (Fig. 2.6) showed that the most important variables for Max_cat were native woodland and annual average temperature with Max_cont also selecting those as the most important variables although in the reverse order. Furthermore, for both models native woodland also seemed to include the most information that is not present in the other EGVs, i.e., the most uncorrelated information without which the model would lose the most quality.

2.3.3 Model evaluation

Model predictions were significantly different among all models after Bonferroni corrections for multiple comparisons ($P<0.001$, two-tailed Wilcoxon signed rank test, paired by model). This is especially interesting given that mean habitat suitability values for Max_cat and Max_cont were very similar in the training data and very different in the presence test data (Table 2.2). Also of note is that

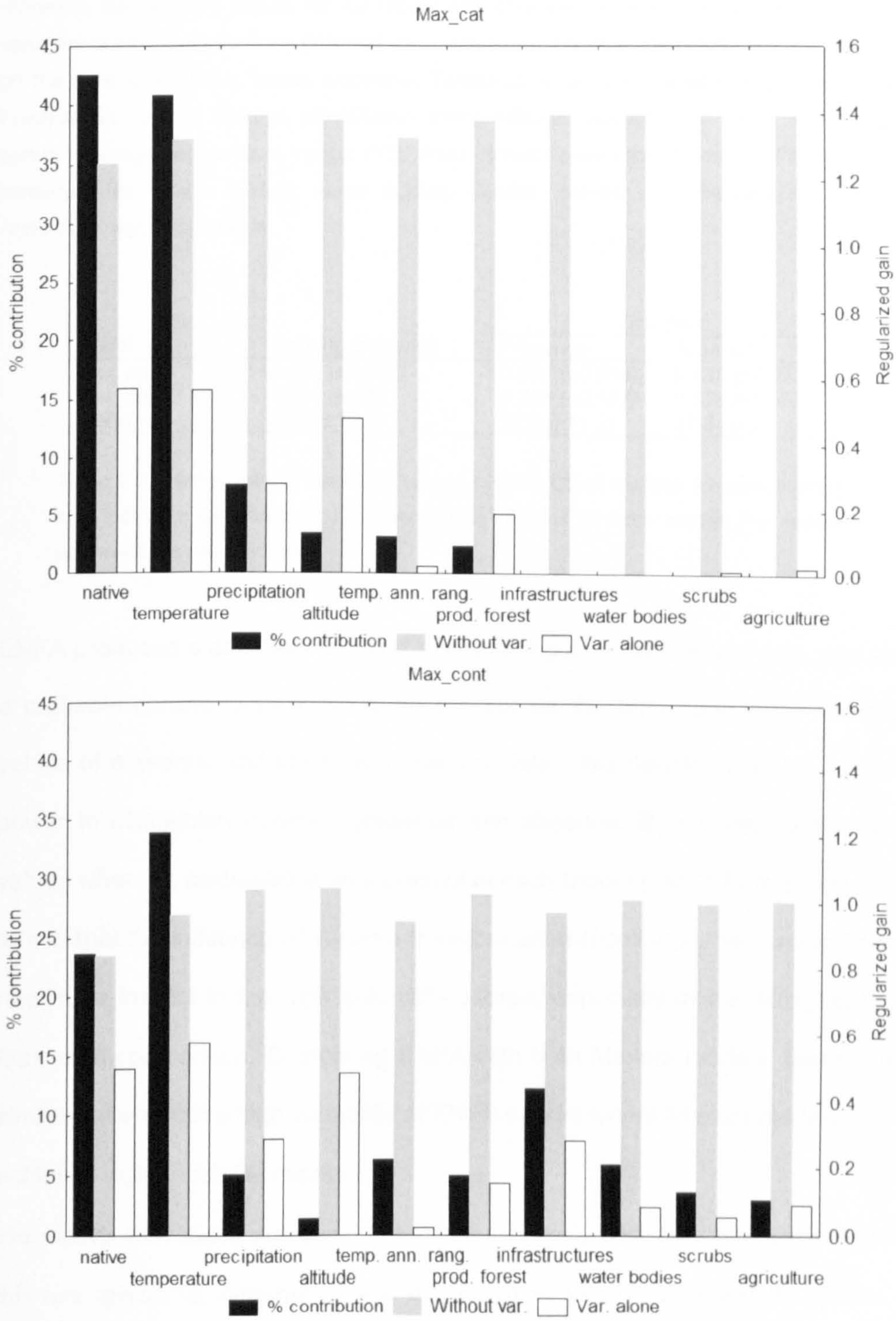

Figure 2.6 – Representation of each variable's importance for the Maxent models (see Methods for abbreviations). The percentage of contribution of each variable to the model is represented by the black bar and corresponding values may be found on the left axis. The other two bars

represent the jackknife results for the model with only one variable (Var. alone) or with all variables but the analysed one (Without var.). Values for the jackknife results are represented on the right axis. Native: native woodland; Temperature: annual average temperature (ºC); Precipitation: annual average precipitation (mm); Altitude: altitude (m); Temp. ann. rang.: annual average temperature range (ºC); Prod. forest: production forests; Infrastructures: infrastructures; Water bodies: water bodies; Scrubs: scrubs and regenerating forests; Agriculture: agriculture fields.

		Test data	
Model	*Training presence*	*Presence*	*Absence*
Max_cat	*0.642 (±0.132)*	*0.592 (±0.096)*	*0.135 (±0.073)*
Max_cont	*0.650 (±0.1)*	*0.328 (±0.107)*	*0.108 (±0.05)*
ENFA	*53.2 (±16.2)*	*36.9 (±11.2)*	*27.8 (±8)*

Table 2.2 – Mean habitat suitability values (± 95% CI) of training (presence only) and test data (presence and absence) with respect to each model. For model abbreviations see Methods.

ENFA produced a decrease in value from training to presence test data, and as a probable consequence there is an overlap on the 95% confidence interval values of presence and absence in the test data. This denotes ENFA's lack of power to distinguish between presence and absence. By comparing the cell values where *B. barbastellus* was present in each model (Fig. 2.7), it is possible to see that the influence of variable transformation (continuous vs. categorical) had more impact in the high suitability scores, especially in the Max_cat vs. Max_cont comparison. Comparing ENFA with both Maxent models, there is a similar pattern with a high variability of ENFA scores where Maxent models only achieved high suitability results.

The models were highly successful in locating previously undiscovered populations of this rare species. *B. barbastellus* was discovered in 15 new areas and the species' known distribution in Portugal was extended by approximately 100 km to the south (Fig. 2.2). Of these 15 new locations, 13

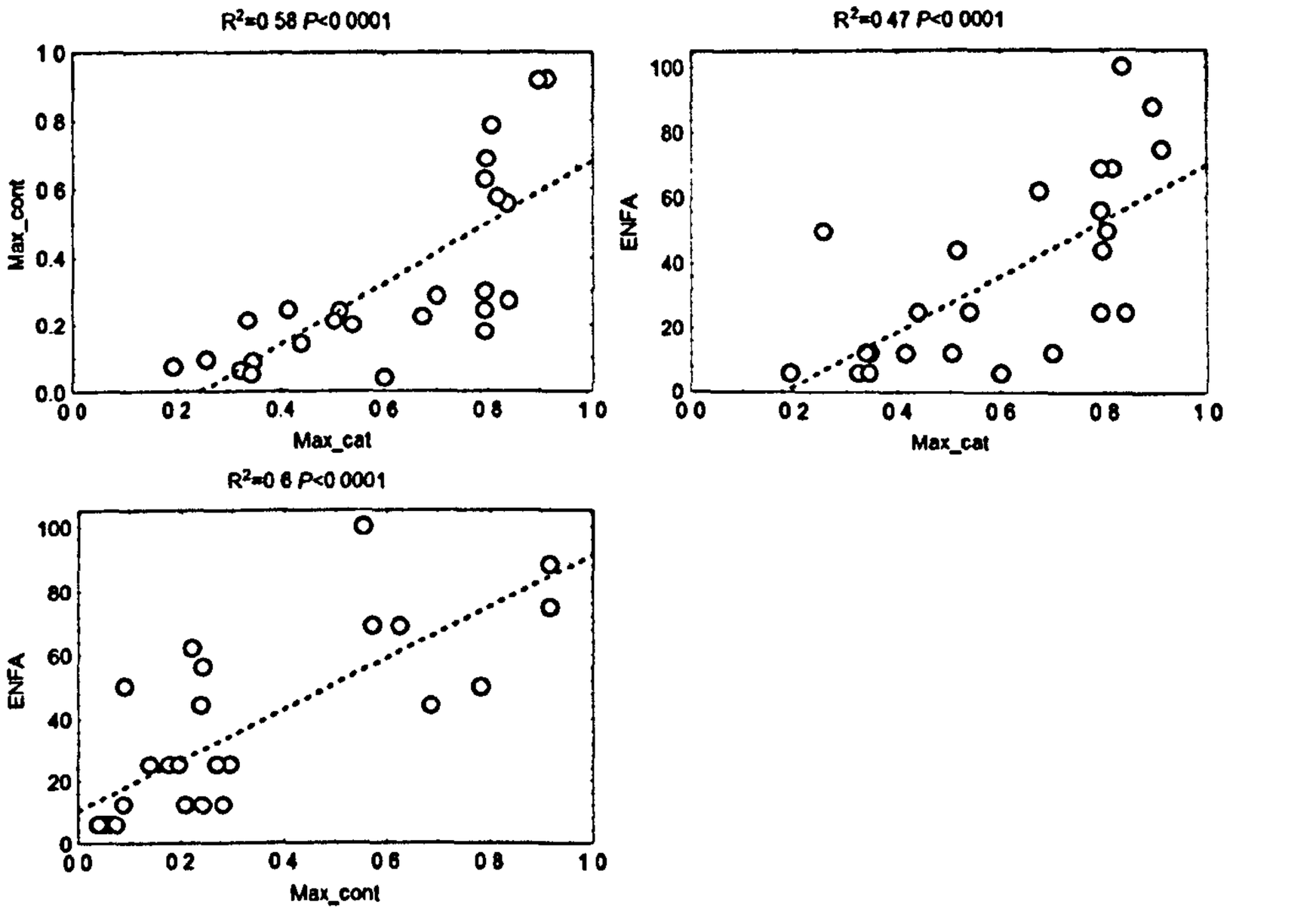

Figure 2.7 – Comparison between each model cell value where *B. barbastellus* was confirmed present. Diagonal dashed line shows the linear regression between the two models. Model abbreviations may be found in Methods.

were predicted by Max_cat, 11 by Max_cont and seven by ENFA. Two detected populations occurred in areas predicted to be of low suitability by all the models. The discrepancies in model's success occurred in the southern part of Portugal, where ENFA predictions were not in agreement with the new findings. Conversely, both Maxent models achieved a similar performance: Max_cat predicted the location of six new southern locations while Max_cont predicted five.

2.3.4 Threshold independent evaluation

By examining the ROC plot, Max_cat was the model with the best performance, although the curve from Max_cont showed a similar pattern (Fig. 2.8).

Consequently, AUC scores were high for those models with ENFA registering a poor score (Table 2.3). Additionally, *B. barbastellus* presence/absence was also significantly correlated with all models although only Max_cat achieved a high score. For the other two models the correlation value was almost half of the Max_cat reflecting the high dispersion and variability in the cell values where *B. barbastellus* presence was confirmed (Fig. 2.8).

2.3.5 Threshold-dependent evaluation

Max_cat was the only model where almost all presences were recorded in cells with values higher than the defined threshold for presence (Fig. 2.9). As stated before, for the other two models the presence values were rather dispersed. On the other hand, for models using only continuous variables almost all of the absences (or unconfirmed presences) were recorded in values below the aforementioned threshold. Nevertheless, even for Max_cat all absences were recorded in cells with values less than 0.4. Considering evaluation indices dependent on the defined threshold, again the performance rank is maintained with Max_cat being the best and ENFA registering the lowest scores in Kappa statistics, correct classification rate and sensitivity. Regarding the models' performance to correctly detect absences (expressed by specificity), all models obtained good results, with Max_cont achieving a very high score while Max_cat and ENFA behaved identically.

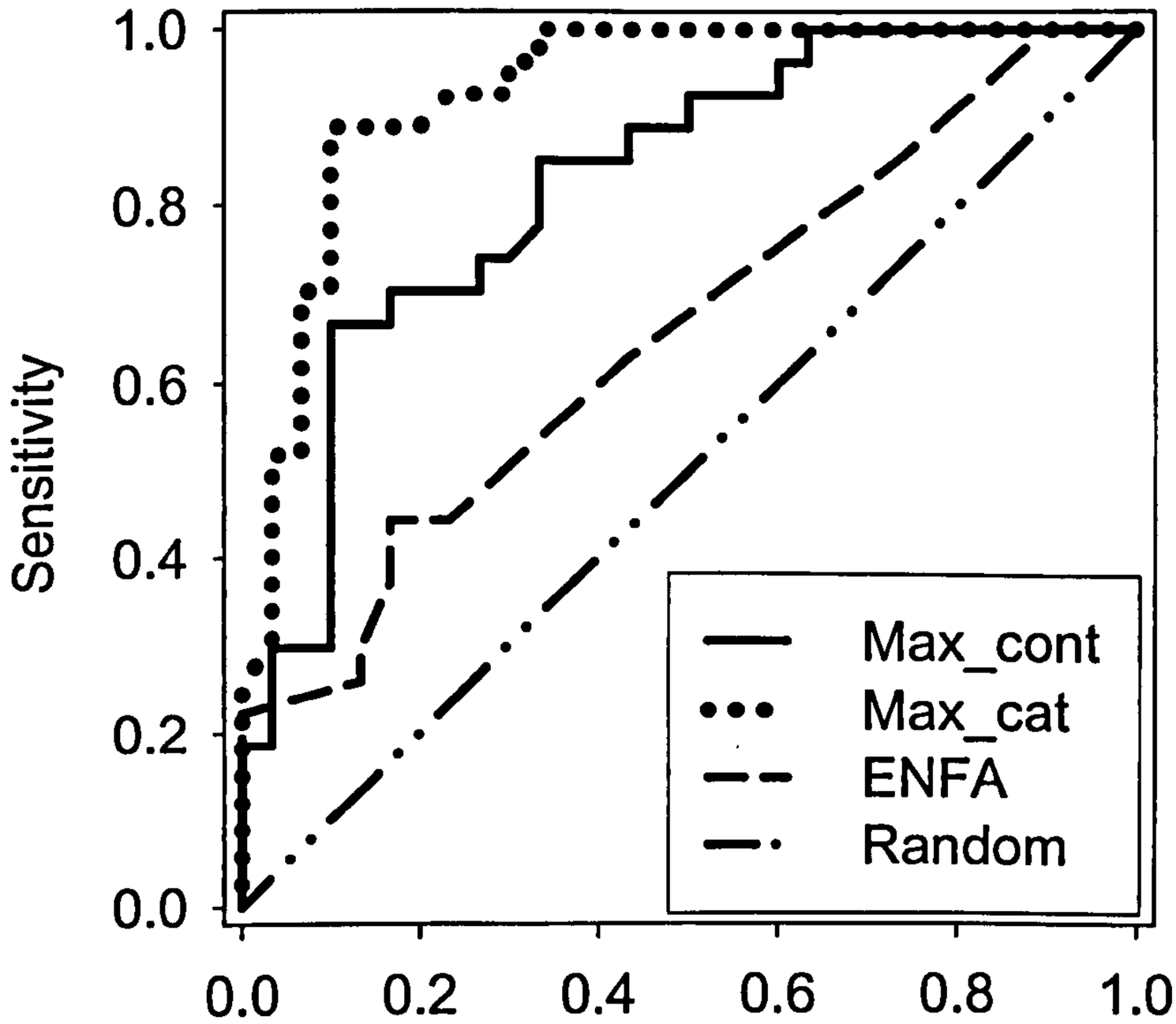

Figure 2.8 – Receiver Operating Characteristics plot for each model (see abbreviations in Methods) using the results from the acoustic transects. The diagonal line represents a random prediction.

	Threshold independent		*Threshold dependent*			
Model	*AUC*	*COR*	*Kappa*	*Correct classification*	*Sensitivity*	*Specificity*
Max_cat	*0.930**	*0.727**	*0.853*	*0.859*	*0.963*	*0.767*
Max_cont	*0.827**	*0.472**	*0.742*	*0.751*	*0.519*	*0.967*
ENFA	*0.614**	*0.312**	*0.612*	*0.614*	*0.444*	*0.767*

Table 2.3 – Comparison of model results with presence/absence test data. For all statistics, the value of one is the best achievable result. For model abbreviations see Methods. * indicates $P<0.05$.

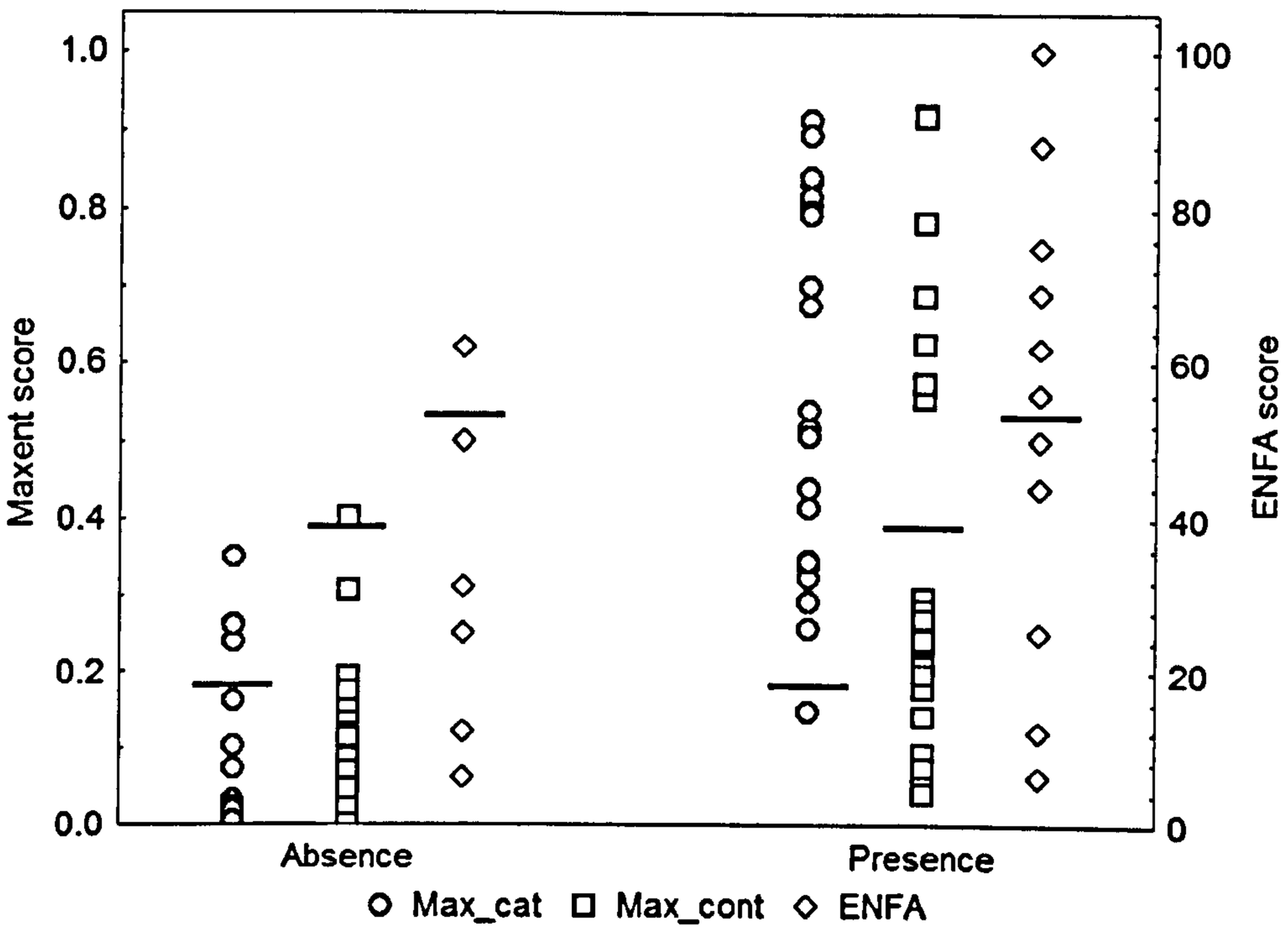

Figure 2.9 – *B. barbastellus* confirmed presence or suspected absence from the acoustic transects with respective model score (see model abbreviations in Methods). Each symbol may correspond to more than one transect. Horizontal lines represent threshold values for each model above which the species is assumed to be present.

2.4 Discussion

2.4.1 Predictive modelling accuracy

The models proved successful in the discovery of new barbastelle populations outside of the known range, even when there was a sampling bias in the presence data. Nevertheless, predictive modelling should be used with care when projecting results to new areas. In fact, model accuracy could be compromised when projected to values outside the range that it originated from (Peterson *et al.* 2007). Sampled data should cover the range of a species' ecological conditions so that the modelled niche can approximate to the realized niche (Wisz *et al.* 2008). Yet, one of the most unique applications

habitat suitability modelling provides is to give insights into new areas in which a species could occur (Pearson *et al.* 2007) or to forecast how distributions might be affected by new combinations of biotic or abiotic factors (Guisan & Thuiller 2005). In fact, some of the most valuable uses for modelling have been to evaluate the impact of climate change on the distribution of species (Guisan & Thuiller 2005), to predict past distributions (Hugall *et al.* 2002) or even to assess the distributions of newly described cryptic species (Raxworthy *et al.* 2003). However, it is important to question whether ecological niche modelling does necessarily predict distribution (Hirzel & Le Lay 2008). The geographical distribution of a species could be limited by several factors that usually lay outside the scope of species distribution modelling, for example, existence of geographical barriers, limited dispersal abilities and biotic interactions (Pearson & Dawson 2003; Phillips & Dudík 2008; Hirzel & Le Lay 2008). For species with a high potential for dispersal, such as bats, most of these factors would probably have less relevance on the ability to colonise potential habitats. Hence, the difference between realized and potential niches may be much smaller when dispersal limitations are accounted for (Hirzel & Le Lay 2008). The quality of the results could have been different at a different spatial scale, geographical area or simply with a different modelled species. However, for species with similar ecological characteristics (rare, high dispersal potential) it is expected that model quality remains similar. In fact, models generated for specialist species tend to have greater accuracy and predictive power than those developed for generalists (Segurado & Araújo 2004; Elith *et al.* 2006) even in cases where the data quality for specialists is poor (Brotons *et al.* 2004). A specialist species usually occurs in a more restricted range of values

within an ecological factor than a generalist species. Hence, it is more likely that fewer data can cover much of the species' realized niche (Segurado & Araújo 2004).

The most recently developed modelling techniques (e.g. Maxent) outperform more established methods like GARP, GLM or Bioclim (Elith *et al.* 2006). Maxent especially has achieved a robust performance showing a good accuracy with low sample sizes and an excellent predictive ability (Hernandez *et al.* 2006; Pearson *et al.* 2007; Wisz *et al.* 2008). Several authors (Hernandez *et al.* 2006; Wisz *et al.* 2008) have suggested for samples sizes <10 Maxent may be one of the best modelling techniques available. My results are in agreement with those studies, because Maxent performed with great accuracy with a small and biased dataset. Max_cat did not predict broad areas of distribution for the *B. barbastellus*. However, these bats were always found in high suitability areas, even in the south of Portugal that were clearly outside the range of the training data. As such, Maxent proved to be robust to sampling bias at this scale which allowed the discovery of new populations and the extension of known distribution.

Zaniewski *et al.* (2002) showed that ENFA produced accurate results when using presence-only data although ENFA is recognized to have a tendency to overestimate the spatial extent of distributions, especially on the periphery of ranges (Brotons *et al.* 2004). This study results showed a similar pattern. A broad and accurate area of high-suitability was identified in the central and northern regions of Portugal but ENFA was very inaccurate in its predictions outside the geographical range of the training data.

2.4.2 Comparison between ENFA and Maxent performance

It is important to understand why these two techniques yielded such a different extrapolation results. Tsoar *et al.* (2007) concluded that more complex techniques (in this study Maxent) are better predictors that the simple ones since they establish more flexible relationships between the dependent and independent variables. In fact, models that have no pre-defined shape of response curves can build models closer to the training data such as those based on smoothing techniques, like Maxent (Randin *et al.* 2006). On the other hand, parametric methods like ENFA are limited by the normal distribution making them more sensitive to bias or extrapolations (Elith *et al.* 2006; Randin *et al.* 2006). Overall, Maxent seems prone to overfitting presence data (Peterson *et al.* 2007) hence more likely to develop omission errors or false absences (the species exists in low suitability areas) while ENFA seemed to have greatest problems in reducing the commission error rate or false positives (predicts occurrence where the species does not exist) in areas outside the range of the training data.

It should also be stated that it is likely that models developed in Portugal might not work in other parts of Europe. Portuguese populations are in the edge of the species' range, and consequently ecological conditions where barbastelles exist in Portugal may be not representative of the species' ecological optimum. Nevertheless, several studies support the theory that niche positions are more than regional phenomena, because a species can occupy similar niche positions in different regions (Randin *et al.* 2006). In fact, the distribution of *B. barbastellus* predicted by Maxent was mainly limited by the availability of native woodland within a range of temperature values. It has been shown that *B.*

barbastellus is dependent upon mature woodland for roosting (Russo *et al.* 2004; Russo *et al.* 2005) and foraging (Kerth & Melber 2009; Hillen *et al.* 2009), where their main prey, moths, are more abundant (Sierro 1999). My results reinforce the need to protect these mature woodland habitats for effective conservation of this threatened species. On the other hand, the more important EGVs determined in ENFA were not directly related to this habitat. Regarding land cover variables, ENFA predictions were mainly influenced by habitats actively avoided by *B. barbastellus* (like production forests and infrastructures). Higher altitudes seem to be highly relevant in the model, but could be correlated with the presence of native Atlantic forest found mainly in central and northern Portugal and typically confined to isolated areas in high mountains where the majority of the training data were located. Altitude by itself is probably not a limiting factor for *B. barbastellus* distribution (as the fieldwork results have shown) but its selection could be a product of sampling bias. This selection of variables could explain why ENFA failed in predicting *B. barbastellus* occurrence in the south while being accurate in the remainder of the country. The combination of abiotic factors in the south clearly differs from the rest of the country although the area of native woodland (in this case oak savannah-like woodland) is vast. By choosing variables different from the ones chosen by Maxent, the location of ENFA high-suitability areas in the south was totally different.

Furthermore, I acknowledge that absence data may contain some false absences (unconfirmed presences) that could have a potential impact on the evaluation statistics. Nevertheless, I believe that results are consistent over the sampling area and so the impact of that error on model predictions does not

justify the differences in their performance. In fact, *B. barbastellus* was discovered in 15 new areas and the species' known distribution extended by approximately 100 km to the south.

2.4.3 Conservation implications

In conclusion, the models proved to be very useful tools for defining suitable areas and for the subsequent discovery of new populations of a rare species over a relatively large geographic scale. A similar approach to other rare species could also be highly productive when addressing issues such as planning field surveys, defining reintroduction areas, identifying population fragmentation, planning rapid biodiversity assessments in poorly documented regions and supporting a wide range of conservation strategies or policies (Engler *et al.* 2004; Hirzel *et al.* 2004; Sattler *et al.* 2008). Nevertheless, I should stress that models are only an approximation to reality hence they can never replace a detailed, ongoing collection of field data (Hirzel & Le Lay 2008). Nonetheless, some studies have successfully used predictive modelling to determine species' conservation status (Sattler *et al.* 2008) or even to find new populations of cryptic species (Raxworthy *et al.* 2003). With enhanced computer processing and the increase of available geographical data, surely new applications will widen the scope of species distribution modelling.

CHAPTER 3

Integrating molecular ecology and predictive modelling: implications for the conservation of the barbastelle (*Barbastella barbastellus*) in Portugal

Abstract

1. In this chapter multiple approaches (population genetics, past and present predictive modelling) were employed to determine the population structure and the potential distribution in Portugal of the Barbastelle, *Barbastella barbastellus*, one of the most endangered European bats.

2. Population structure was analysed by sequencing two mitochondrial DNA fragments: cytochrome-*b* and D-loop. The latter fragment showed almost no variation with only three haplotypes found. In contrast, 13 haplotypes were identified in cytochrome-*b* sequences although without significant population structure, despite the existence of unique haplotypes in the northern and southern regions.

3. Predictive modelling for the current conditions showed that *B. barbastellus* distribution is fragmented and highly dependent on the existence of native woodland, with the majority of the suitable areas located in the centre and north of the country, while the predicted occurrence in the south was very restricted. Bioclimatic models for the Last Glacial Maximum also exhibited a similar pattern which, in agreement with the location of the unique haplotypes, suggested that some degree of isolation has existed between northern and southern populations. Nevertheless, the separation of these populations is not significant in the genetic analysis undertaken.

4. *Synthesis and applications:* Portuguese populations should be managed as a single unit. The maintenance of the existing mature native woodland is paramount for conservation. In conclusion, this study illustrates how combining genetic analysis with predictive modelling can help conservation management of a poorly documented species in a rapid and low cost manner.

3.1 Introduction

Rare species are a conservation priority but their management is frequently complex, requiring studies with multiple approaches, such as demography, population genetics and ecological modelling (Gebremedhin *et al.* 2009). Unfortunately, it is usually difficult to obtain quality data for the adequate development of broad-scale conservation strategies, with the majority of studies focusing only on a limited set of aspects. Rare species are often characterized by high levels of population fragmentation, occupy a restricted area and, frequently exhibit low effective population sizes (Drury 1974). Rare species are also characterized by a great lack of knowledge due to their scarcity or ecological characteristics that make them difficult to detect or to collect data on (e.g. Palma *et al.* 1999; Chadès *et al.* 2008). On the other hand, the difficulty of detecting a species may sometimes give a misleading impression of rarity, when in fact the taxon could be more abundant than expected (Ciechanowsky *et al.* 2007).

Over recent years several promising methodologies have emerged that produce powerful outcomes without the need of collecting huge amounts of data. The advent of molecular techniques has greatly contributed to conservation management, especially with the use of mitochondrial DNA (mtDNA) analyses that are fast to execute and low in cost. Understanding the patterns and causes of a species' genetic structure is crucial for the identification of past historical events (e.g. bottlenecks, population expansions), the identification of sub-populations and Management Units, i.e. populations that exchange so few migrants that they become demographically independent (Avise 2000), or unique gene pools that by themselves are priorities for

conservation protection (Moritz 1994). This way, conservation efforts can be directed more efficiently to maintain population viability and genetic variability (Michaux *et al.* 2004). However, these types of results only allow the determination of a species' population structure for the sampled geographical area. The application of genetic data *per se* on a spatial level only reveals discrete spatial patterns - hence the geographical edges of populations and where they are (or were) located are not spatially defined (e.g. Salgueiro *et al.* 2003; Ruedi *et al.* 2008).

Recent developments in species distribution modelling can also help with filling that knowledge gap. Predictive modelling already been proven to be a powerful tool for inferring the potential distribution of a species (Greaves *et al.* 2006) and some techniques are robust enough to use presence data only, even when data are scarce and collected in a non-systematic ways (Brotons *et al.* 2004; Elith *et al.* 2006; Hernandez *et al.* 2006; Hernandez *et al.* 2009). Moreover, because population structure may result from both current and historic events, it is also important to consider the roles of past climatic conditions in determining geographic ranges in the past (Lamb *et al.* 2008). Recently, the application of predictive modelling to past conditions has helped in the determination of a species' potential distribution in events like the Last Glacial Maximum (LGM) (Hugall *et al.* 2002; Waltari *et al.* 2007). The application of this technique together with genetic analysis could allow a better understanding of population structure (Lamb *et al.* 2008; Gebremedhin *et al.* 2009).

I studied one of the rarest European bat species, the barbastelle *Barbastella barbastellus* Schreber, 1774. This tree-dwelling bat is widespread in Europe although its distribution is highly fragmented (Mitchell-Jones *et al.* 1999). It

seems to be dependent upon native mature woodland and hence deforestation and habitat fragmentation are probably associated with its suspected population decline (Russo *et al.* 2004). In this study I focused on the *B. barbastellus* populations of Portugal where it was first discovered in the country in 1980 (Palmeirim 1990) and until recently was known to occur in only a few locations. Therefore, this situation involves a rare species of conservation priority with a great lack of knowledge and no local management strategy to address this species' conservation needs. As such, this work may constitute an example of how to diminish existing knowledge gaps with a fast and low cost approach that could allow the development of novel conservation measures. I combined the use of mtDNA analysis with predictive modelling in order to: (1) to make a first assessment of the population genetic structure of *B. barbastellus* in Portugal; (2) to predict the current potential distribution of *B. barbastellus* and determine which ecological factors are limiting it; (3) to investigate population distribution and demographic history of *B. barbastellus* since the LGM; and (4) to integrate genetic analysis with predictive models for a better conservation management of the species in Portugal.

3.2 Methods

3.2.1 Study area and sampling

The study was carried out in mainland Portugal (approximately between coordinates 36°N - 41°N and 7°W - 9°W), where two major biogeographic zones divide the country. In the north and the majority of the central regions the Atlantic temperate climate dominates with mild summers and cold, rainy winters. The landscape is mountainous with native forests mainly composed of

oaks (*Quercus robur*, *Q. pyrenaica*, *Q. faginea*), chestnut (*Castanea sativa*), birch (*Betula* spp.), ash (*Fraxinus* spp.), and other flora that constitute a typical European Atlantic forest. The southern regions are characterized by a Mediterranean climate with mild winters and hot, dry summers. As a consequence, the landscape is dominated by an oak savannah-like woodland (*Quercus suber* and *Q. ilex*) spreading over vast plains (Sillero 2009).

Bats were captured during mist-netting sessions between May to October of 2005-2007 in areas where predictive models from a previous study (unpublished data) indicated a high probability of occurrence. Based on the predicted distribution, four regions within Portugal were defined for sampling: Northwest (NW), Northeast (NE), Centre (C) and South (S). In order to guarantee the sampling of independent populations, sampled regions were more than 100 km apart (Fig. 3.1); distances between sampling regions were determined such that the reported home range and migration distance were exceeded (Greenaway 2001; Riede 2001). Tissue samples were obtained from a nonlethal, 3 mm sterile biopsy punch of the wing membrane (Worthington Wilmer & Barratt 1996) and were preserved in 96% ethanol.

3.2.2 Genetic assay

Genomic DNA was extracted from half wing punches using an E.Z.N.A. Tissue DNA kit (Omega Bio-Tek, GA, USA), eluted and stored in 50 µl of the provided elution buffer. Two mitochondrial fragments were amplified: the cytochrome-*b* (*cyt b*) and the hypervariable domain (HVII) of the D-loop. To amplify these fragments the following sets of primers were used: Bat_*cytb*_1 (Li *et al.* 2006)

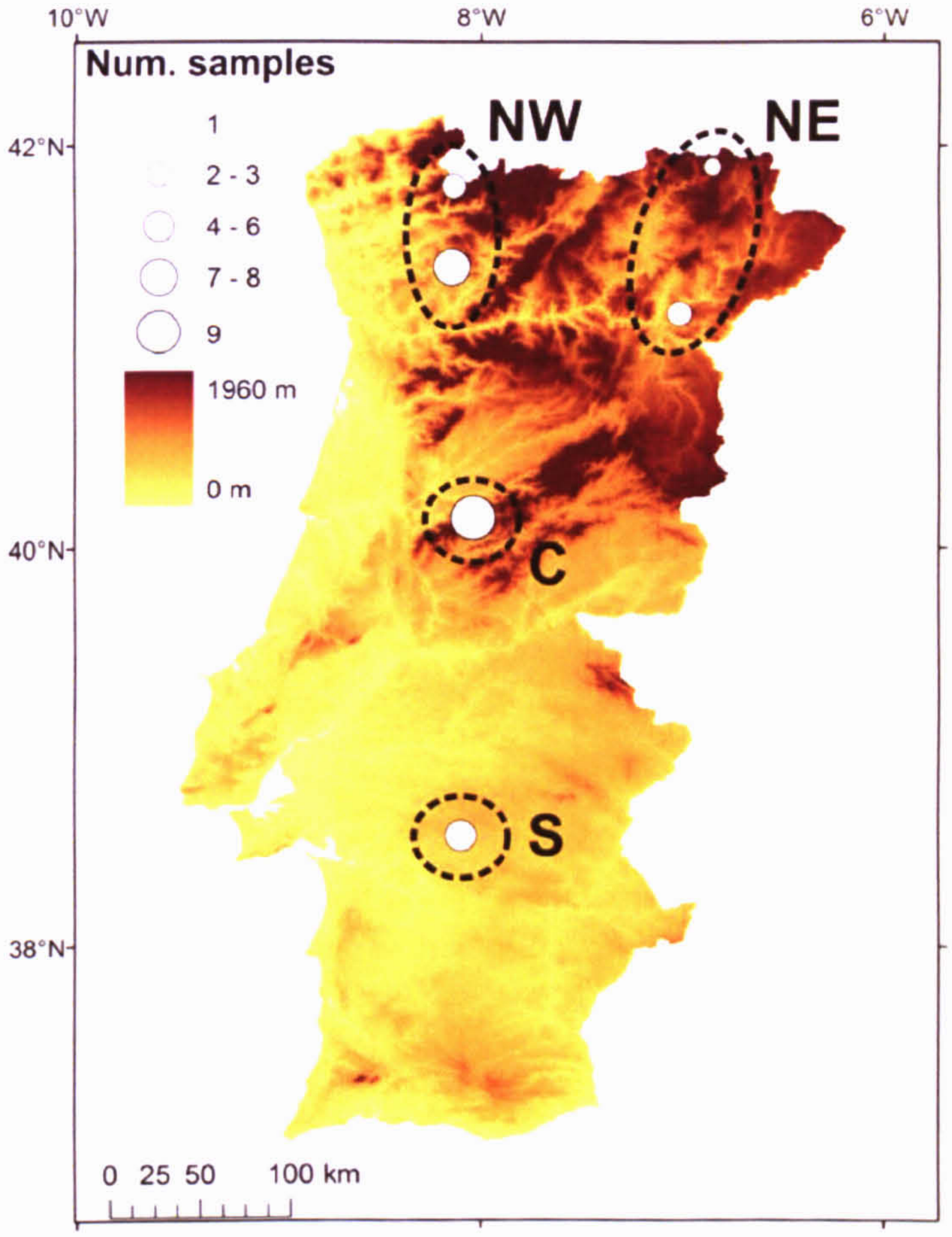

Figure 3.1 – Location of samples used for genetic analysis. Circle area is proportional to sample size. Due the scale used each circle may contain more than one sampling site. Defined sampled regions are represented by dashed ellipses.

and Bat_*cytb*_2 (Zhang *et al.* 2007) for *cyt b* and L16517 (Fumagalli *et al.* 1996) and sH651 (Castella *et al.* 2001) for D-loop. Polymerase chain reaction (PCR) for *cyt b* was performed in 25 µl reaction volumes containing 5 mM $MgCl_2$, 0.24 µM of each primer, 0.6 µM of each dNTP, 1 unit of Taq DNA polymerase (QIAGEN, CA, USA) and 5 – 10 ng of genomic DNA. The amplification consisted of an initial denaturation at 95ºC for 5 min, followed by 39 cycles at 95 ºC for 40 s, 52ºC for 45 s, and 72ºC for 80 s, with a final

elongation step at 72°C for 10 min. The amplified fragments of *cyt b* were sequenced on an automated sequencer (ABI 310; Applied Biosystems) in both directions using the same primers. The overlapping fragments of *cyt b* were then assembled to produce sequences of 1140 bp. Regarding PCR conditions for the D-loop, the procedure described by Castella *et al.* (2001) was followed. The amplified fragments were sequenced in one direction using primer L16517 producing a sequence of 297 bp. Sequences from both fragments were examined, edited and aligned using the software Bioedit v.7.0.1 (Hall 1999). All sequences are to be submitted to GenBank.

3.2.3 Genetic analyses

The levels of genetic diversity within each region and for the whole country were described as haplotype (h) and nucleotide (π) diversities and genetic differences (d). Genetic differentiation among populations was quantified by performing a global test of differentiation among samples. All calculations were done using DNAsp v.4.50.3 (Rozas *et al.* 2003).

To test for geographical genetic structure, an analysis of molecular variance (AMOVA) with 10 000 permutations was used. In addition, pairwise Φ_{ST} values to assess the amount of haplotypic subdivision were computed. To examine the demographic history of *B. barbastellus* pairwise mismatch distributions were plotted and tested them to a model of rapid expansion (Schneider & Excoffier 1999). This analysis compares the observed frequencies of pairwise nucleotide differences to those expected on a model under a single rapid demographic expansion (Rogers & Harpending 1992). One thousand parametric bootstraps were used to generate the expansion model, while the sum of square

deviations (SSD) between the observed and predicted mismatch distributions was calculated in order to determine the proportion of simulations producing larger expected SSD values than observed. In addition, and because population changes are expected to leave detectable patterns in the distribution of pairwise differences, the smoothness of this distribution was measured by calculating the raggedness index (Harpending 1994). To test the hypothesis of demographic expansion, two indices were calculated, the Fu's neutrality statistic *Fs* and the Tajima's *D*. Fu's *Fs* tests the probability of observing a random neutral sample with no more alleles than the observed value of pairwise differences in the sample (Fu 1997). Tajima's *D* tests whether the parameter derived from the average number of pairwise nucleotide differences is equal to the parameter derived from the number of segregating sites in the sample (Tajima 1989). These tests significance was calculated using 10 000 coalescent simulations. All calculations were done in Arlequin 3.1 (Excoffier *et al.* 2006).

Additionally, haplotypes were connected on a network using the 95% parsimony criterion implemented in the program TCS (Clement *et al.* 2000). With this method it was intended to detect potential spatial patterns existing on the haplotype distribution. This technique is particularly suited to the analysis of single species gene genealogies, where ancestral and descendant haplotypes may coexist (Posada & Crandall 2001).

3.2.4 Predictive modelling

For model calculation all known locations of *B. barbastellus* in Portugal (N=47) were used as the dependent variable. Data were provided by several

collaborators, in addition to personal data obtained through acoustic transects done between 2005-2007. Comparing to occurrence data used in the previous chapter, there was a substantial increase in the number of presence data for models' development in this chapter. Moreover, a set of independent ecogeographical variables (EGV) was also considered: annual average temperature (ºC), annual average precipitation (mm), average temperature range (ºC), average temperature of the warmest quarter (ºC), average temperature of the coldest quarter (ºC), altitude (Hijmans *et al.* 2005; WorldClim dataset available at http://www.worldclim.org) and land cover (source Global Land Cover 2000; http://www-gvm.jrc.it/glc2000/ and Instituto Geográfico Português). This latter EGV was reclassified into six classes namely, infrastructures (towns and villages, industrial areas, roads, etc.), agriculture fields including steppes, production forests (mainly conifer and *Eucalyptus* spp.), scrubs and regenerating forest, native woodland and water bodies. Regarding climatic variables, the chosen ones resulted from a set of assays to obtain the best predictive model with uncorrelated variables. Besides the mentioned variables, the following EGVs were also tested and excluded afterwards: maximum temperature of the warmest month (ºC), minimum temperature of the coldest month (ºC) and precipitation of the driest month (mm). Correlations were assessed by a Principal Component Analysis and respective correlation matrix in the software SPSS v15.0 (SPSS Inc., Chicago, Il.), and variables with correlation values higher than 0.8 were excluded from the analysis. All digital information had a resolution of 30 arc seconds (~900m), thus the study area included 133291 cells corresponding to an area of approximately 90100km^2. Additionally, I also calculated bioclimatic models for

the present and for the Last Glacial Maximum (23000 – 18000 years BP) using only the above selected climatic variables. LGM refers to the period of maximum extent of the ice sheets during the last glacial period (Clark *et al.* 1999). Two different General Circulation Models (GCM) were used for this latter period: the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC, version 3.2). Climate simulation procedure and downscaling can be consulted in Waltari *et al.* (2007). Both GCM had a spatial resolution of 2.5 arc minutes (~4.5km) and were downloaded from the WorldClim dataset (Hijmans *et al.* 2005; Waltari *et al.* 2007).

As in the previous chapter, a presence-only technique was chosen for modelling because I do not have reliable absence data and the elusive and nocturnal behaviour of bats adds even more uncertainty to absences. I used a maximum entropy modelling technique (Maxent species distribution modelling, v.3.3.0; http://www.cs.princeton.edu/~schapire/maxent) that has achieved good results on the statistical indices when compared with other modelling techniques (Brotons *et al.* 2004; Elith *et al.* 2006; Hernandez *et al.* 2006; Hernandez *et al.* 2009). Maxent definitions and conception can be consulted in Phillips *et al.* (2006) and in the previous chapter. Models were run with 80% of the presence data while the remaining 20% were used for model testing. Because Maxent randomly chooses which presence data to include in the training or test models, this would imply that produced models would be different according to chosen presence data. Therefore 100 model replications were ran and averaged into a single model (with standard deviation shown when appropriate). Calculations where done in the autofeatures mode with a

maximum of 1000 interactions and regularization set to 0.5. I also calculated the bioclimatic models for present conditions and for the LGM with an identical approach, although using variables with a resolution of 2.5 arc minutes (see above). To verify which variables were the most important for model building, a Jackknife analysis of the gain (a measure of likelihood between the presence data and ecological variables) was made with the training data being results presented in graphics. The relationship between the species and most relevant EGVs was assessed by the analysis of response curve plots obtained with univariate models. The obtained model was then tested with Receiver Operated Characteristics (ROC) plots to evaluate their predictive ability. The area under curve (AUC) of the ROC analysis provides a single measure of the model performance (Liu *et al.* 2005) and ranges from 0.5 (randomness) to 1 (perfect discrimination). The threshold value above which it is considered that the species is present was selected from the ROC plot at the point with values higher than equal training sensitivity plus specificity, while it was considered absent for values lower than 10% of training presence (Suárez-Seoane *et al.* 2008; Lamb *et al.* 2008; Raes *et al.* 2009).

3.3 Results

3.3.1 Genetic diversity and population structure

Thirty samples were amplified successfully for the complete *cyt b* (1140bp) and the D-loop fragment (297bp). This latter fragment had a very low haplotype diversity (h=0.536±0.0482) while nucleotide diversity was π=0.0019±0.0002. Moreover, only three haplotypes were obtained resulting from 2 variable sites corresponding to 1 transition and 1 transversion, and there was only 1

parsimony-informative site. Consequently, overall mean genetic difference (uncorrected p-distance) was d=0.002±0.002 while mean differentiation within the four regions was d=0.002±0.001. In the light of these results, no further analysis was conducted on this fragment due to the lack of variability.

On the other hand, 13 *cyt b* haplotypes were obtained (see Table S3.1 Supplementary material) resulting from 18 variable sites, randomly distributed along the fragment, of which 16 were transitions and 2 were transversions and 16 sites were parsimony-informative. No indels were found and the sequences exhibited a low proportion of guanine residues (A: 0.29; C: 0.29; G: 0.15; T: 0.27). Global haplotype diversity was high (h=0.929±0.022) while nucleotide diversity was moderately low (π=0.00383±0.0004). Differentiation between study regions was low with an overall mean genetic difference (uncorrected p-distance) of d=0.004±0.001 while mean differentiation within each region was d=0.003±0.001. Moreover, Φ_{ST} among regions varied between 0 and 0.4 although without a significant population structure ($p>0.05$), probably due to a high proportion of shared haplotypes among regions. In fact, half of the analysed sequences belonged to four haplotypes distributed among the four study regions. Consequently, analysis of molecular variance revealed that only within populations there was a significant genetic variation, as well as most (86%) of the genetic diversity (Table 3.1).

The parsimony network of haplotype topology (Fig. 3.2) revealed no strong population structure, with the majority of haplotypes separated by only a few mutations. Indeed, several haplotypes are shared between different populations. Haplotypes 1, 5 and 10 were found in both northern and southern populations, while populations in the central region only shared haplotypes (3

Structure	Source of variation	Variation (%)	Fixation indices	*p* value
Four regions	Among regions	1.82	Φ_{CT} 0.018	0.48
	Among populations/within regions	11.9	Φ_{SC} 0.1372	0.55
	Within populations	86.28	Φ_{ST} 0.1211	0.002

Table 3.1 – Analysis of molecular variance among *cyt b* sequences of the barbastelles. For region definition see Methods and Fig. 3.1.

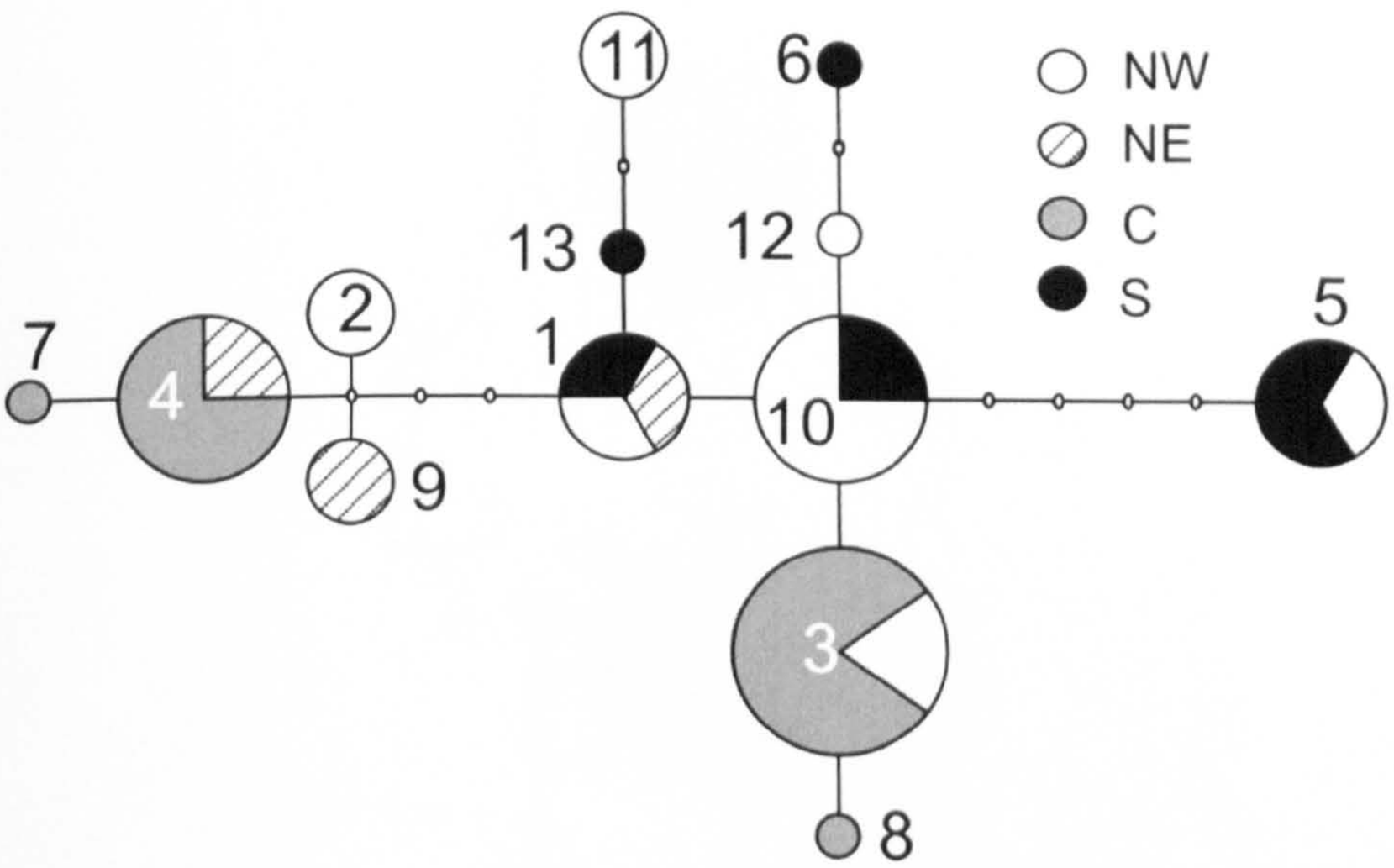

Figure 3.2 – Parsimony network based on the mutational differences among *cyt b* mtDNA sequences found in 30 *Barbastella barbastellus* individuals, based on a total of 13 haplotypes. Each bar represents a mutation event, whereas empty circles refer to unsampled haplotypes. Circle size is proportional to the number of individuals presenting that particular haplotype (numbers identify the haplotype; see Table S3.1m Supplementary material). Shading indicates the proportion of individuals sampled in different geographical regions within the study area.

and 4) with the northern ones (Fig. 3.3). Also of note was that all regions sampled contained unique haplotypes, although those haplotypes in the central populations (7 and 8) derived from the more common ones (4 and 3 respectively) by single mutations.

The model of a rapid demographic expansion did not achieve a significant value (SSD=0.02, $P<0.28$; Raggedness index=0.04; $P<0.36$). Moreover, the mode of the mismatch distribution was shifted towards higher values that

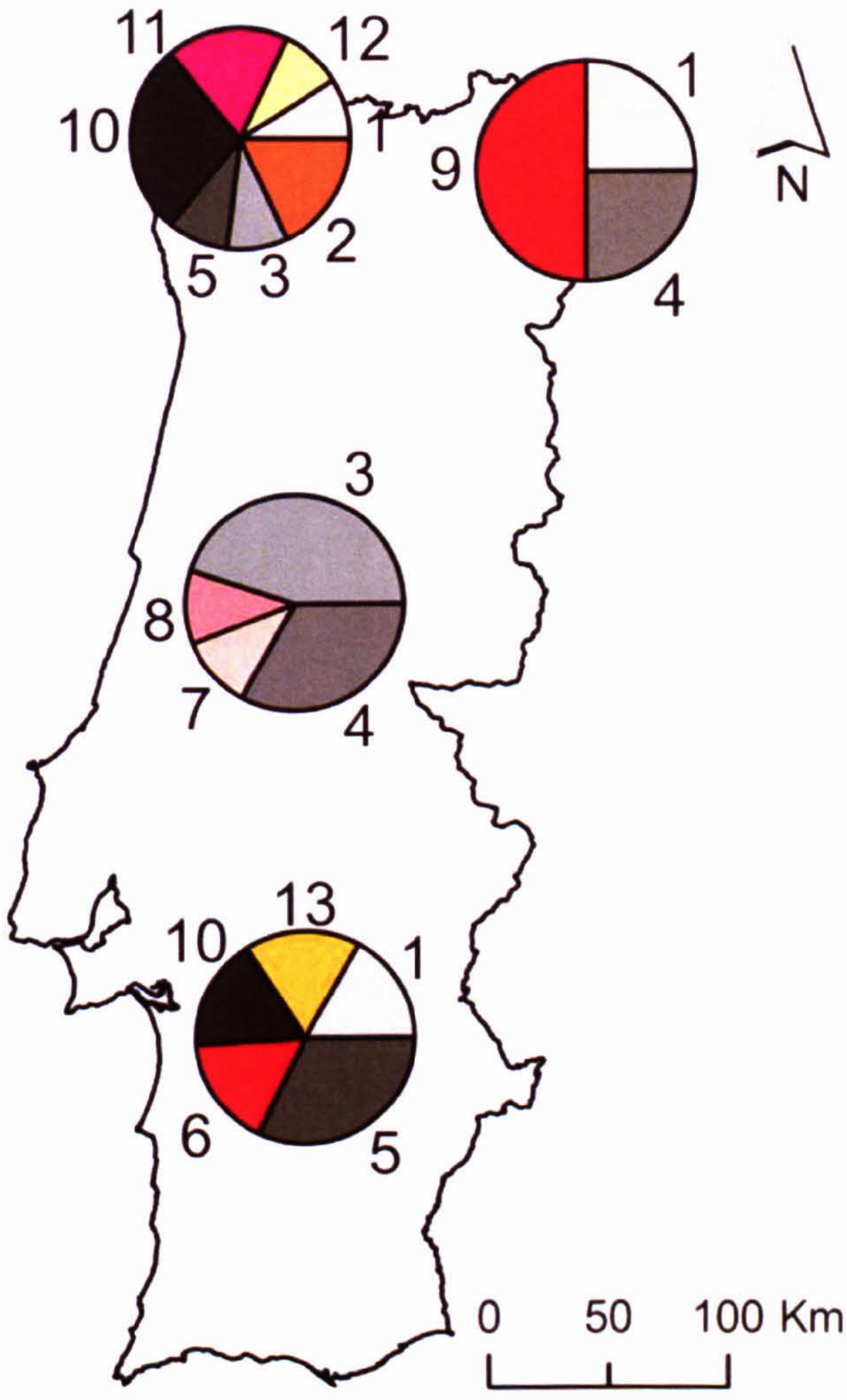

Figure 3.3 – Distribution and haplotype frequency among sampled regions where the numbers identify the haplotype (see Table S3.1, Supplementary information). Unique haplotypes are represented by colours, while shared haplotypes are in a grey scale.

altogether with the multiple peaks observed in the number of pairwise differences, suggesting that populations have remained constant in size (Fig. 3.4). In agreement with this, the more sensitive Fu's *Fs* test (Fs=-2.29, P<0.18) and Tajima's *D* (D=-0.13, P<0.43) gave non-significant results, thus supporting the lack of expansion scenario. Furthermore, the negative value obtained in the

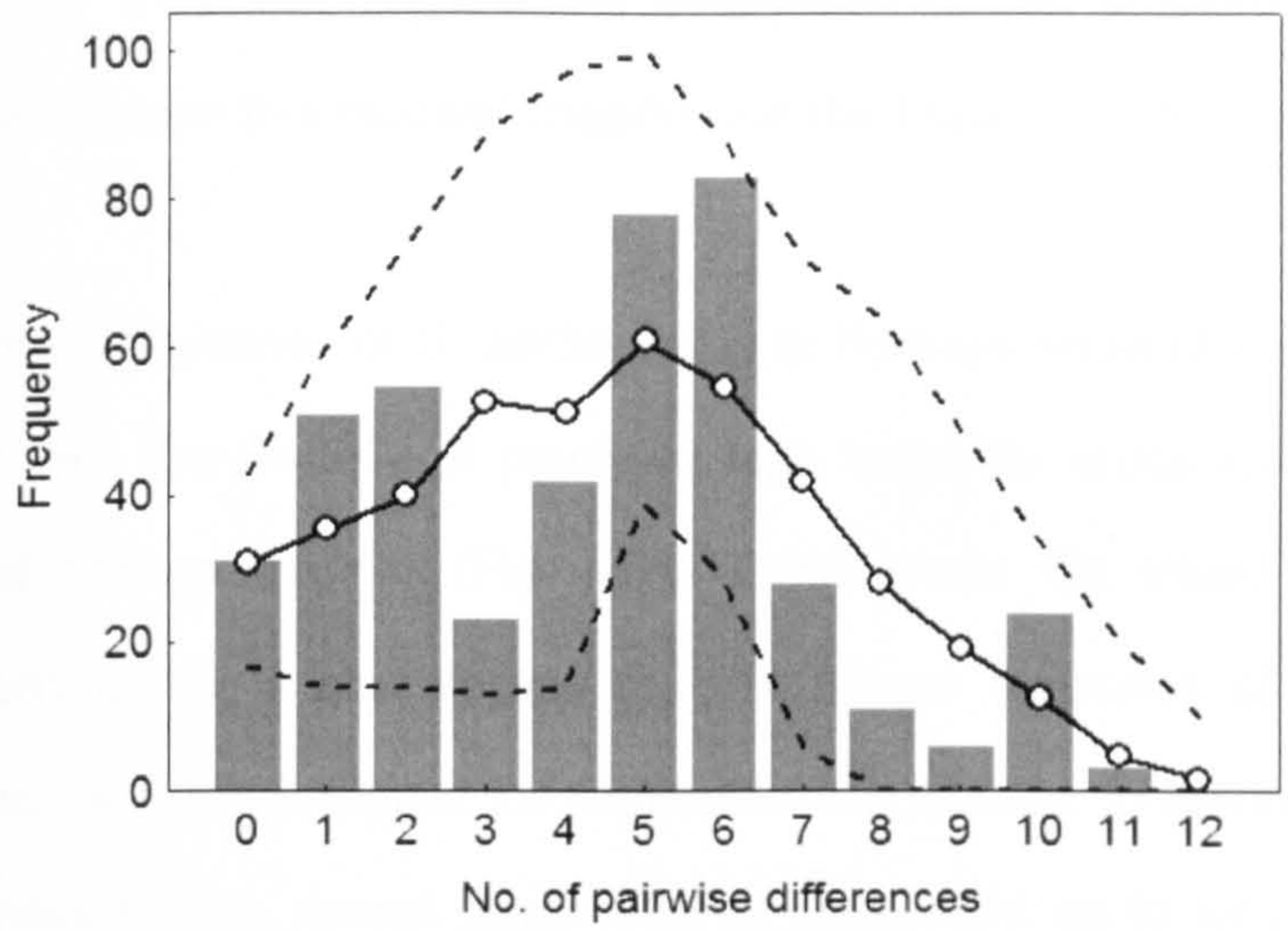

Figure 3.4 – Mismatch distributions of *B. barbastellus* in Portugal. The grey bars show the observed distributions of the pairwise nucleotide differences. The solid line represents the expected distribution under a model of sudden demographic expansion, while dashed line indicate the lower and higher 95% confidence intervals.

Fu's *Fs* statistic suggests that there was an excess of recent mutations or rare alleles in all regions.

3.3.2 Analysis of species distribution models and ecological predictors

All models that predicted present distribution were obtained by averaging 100 models. Models including all variables had very good predictive power for training (average AUC= 0.9 ± 0.01) and test data (average AUC= 0.81 ± 0.06) in spite of values being slightly lower for the latter. Likewise, bioclimatic models showed good predictive power for training data (average AUC= 0.84 ± 0.01), although test data results were slightly lower (average AUC= 0.74 ± 0.07).

Overall, there were no considerable differences in the predicted areas of occurrence between this models' chapter and the Maxent models calculated in chapter 2.

The potential occurrence of *B. barbastellus* in Portugal showed a fragmented distribution with the majority of predicted high suitability areas located in the central and northern regions (Fig. 3.5). Furthermore, the inland south and several parts of the central region contained large expanses of unsuitable areas, hence with low potential for population connectivity. In comparison with the bioclimatic model, overall there is large agreement as to where suitable areas are located (Fig. 3.5). However, a deeper analysis shows that in the north and centre predicted area of occurrence in the bioclimatic model is larger than in the model with all variables, suggesting that factors besides climate are limiting the distribution of *B. barbastellus* in those regions. In contrast, in the south the area of occurrence predicted by the model with all variables extends outside that predicted by climatic conditions alone. Again, this suggests that factors other than climate could be promoting the expansion of *B. barbastellus* beyond suitable climatic conditions.

In fact, land cover was the variable with the greatest contribution to the model that included all variables and the one with the most uncorrelated information, without which this model would lose the most quality (Fig. 3.6). Within this variable, *B. barbastellus* occurrence was clearly associated with Portuguese native woodland and, to a lesser extent, with water bodies and agricultural fields (Fig. 3.7). In addition, *B. barbastellus* presence was also limited by the average temperature of the warmest quarter (Fig. 3.6). This latter variable was

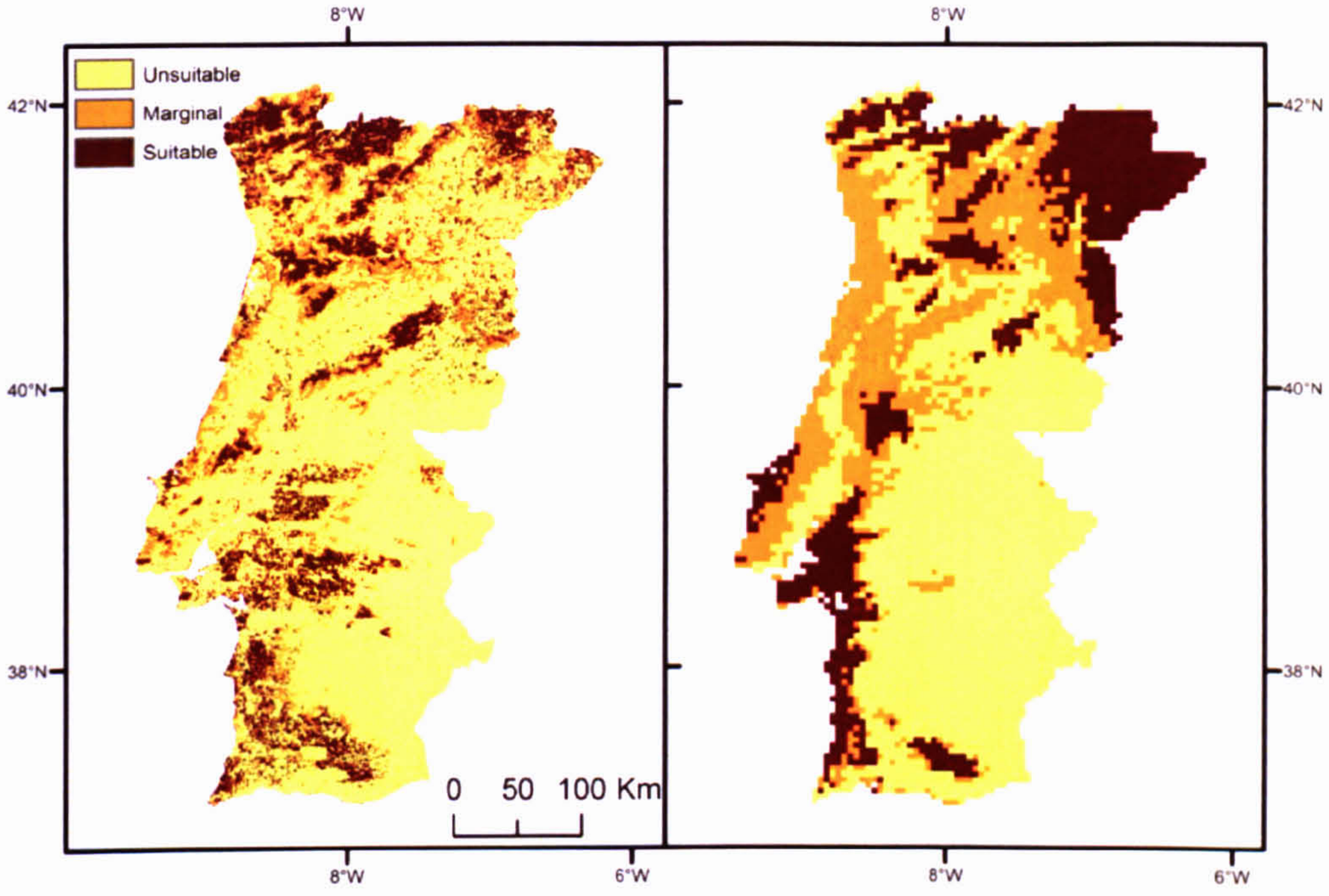

Figure 3.5 – Current potential distribution for *B. barbastellus* in Portugal as computed by Maxent. The left hand figure represents the model with all variables with a 30 arc seconds resolution, while right figure contains only climatic variables with 2.5 arc minutes resolution.

also the most important for the bioclimatic models (data not shown). The relation between average temperature of the warmest quarter and the species presence suggests that higher temperatures are less selected by *B. barbastellus* (Fig. 3.7).

Regarding the models for the LGM, both predicted a large suitable area comprising most of the northern and central regions (excluding the high mountains) with the CCSM model predicting a larger suitable area within those regions (Fig. 3.8). On the other hand, model predictions differed for the southern region where CCSM identified only small three suitable areas while according to the MIROC model a greater area in the southern region had

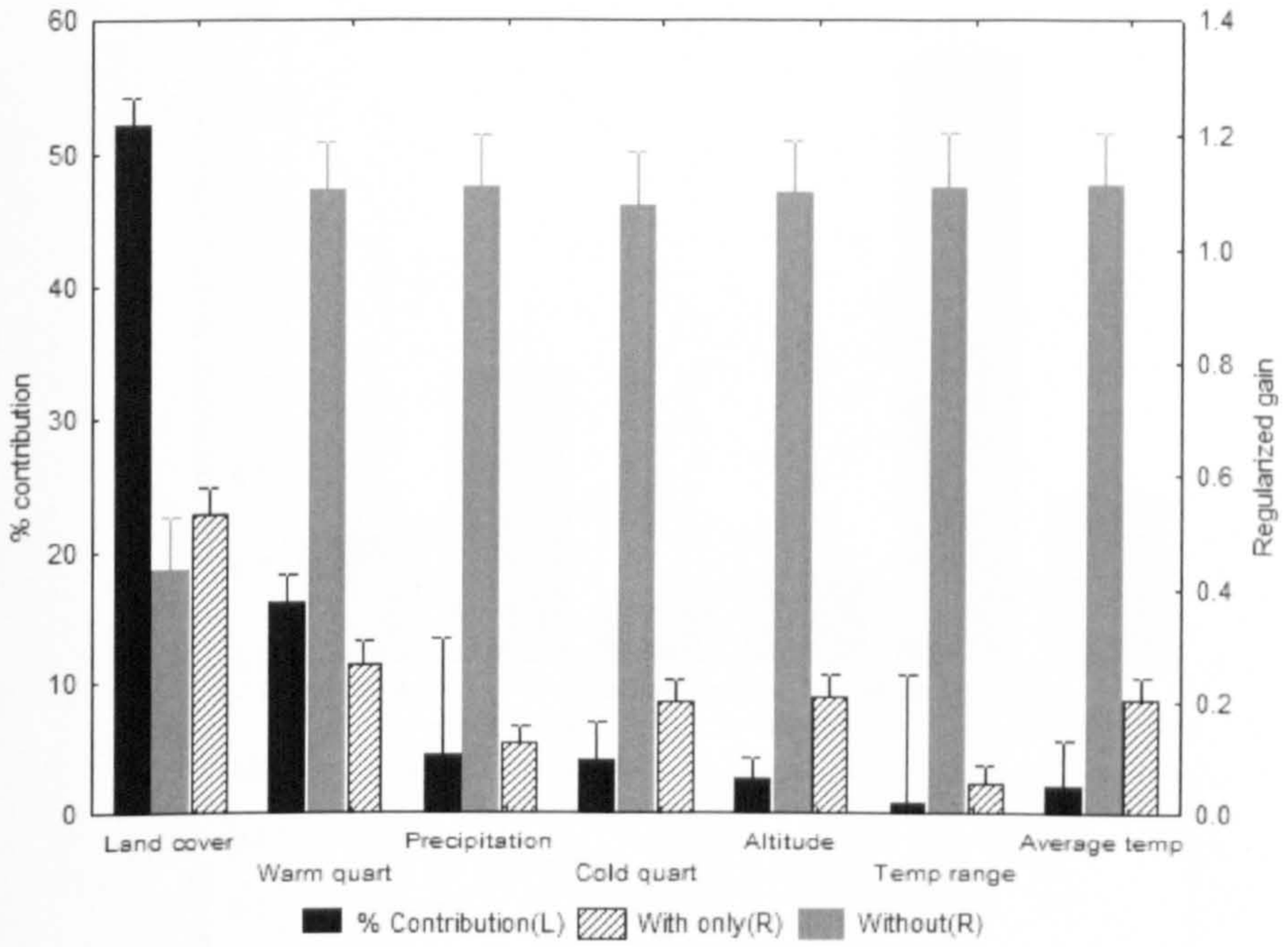

Figure 3.6 – Representation of each variable's importance in the Maxent model where land cover includes the six habitat variables. The percentage of contribution of each variable to the model is represented by the black bar and corresponding values may be found on the left axis (L). The other two bars represent the jackknife results for the model with only one variable or with all variables but the analysed one. Values for these results are represented in the right axis (R). Warm quart: average temperature of the warmest quarter (ºC); Precipitation: annual average precipitation (mm); Cold quart: average temperature of the coldest quarter (ºC); Temp range: average temperature range (ºC); Average temp: annual average temperature (ºC).

suitable bioclimatic conditions for *B. barbastellus*. Nevertheless, both models agreed that it was highly probable that a large unsuitable gap existed between the south and central populations.

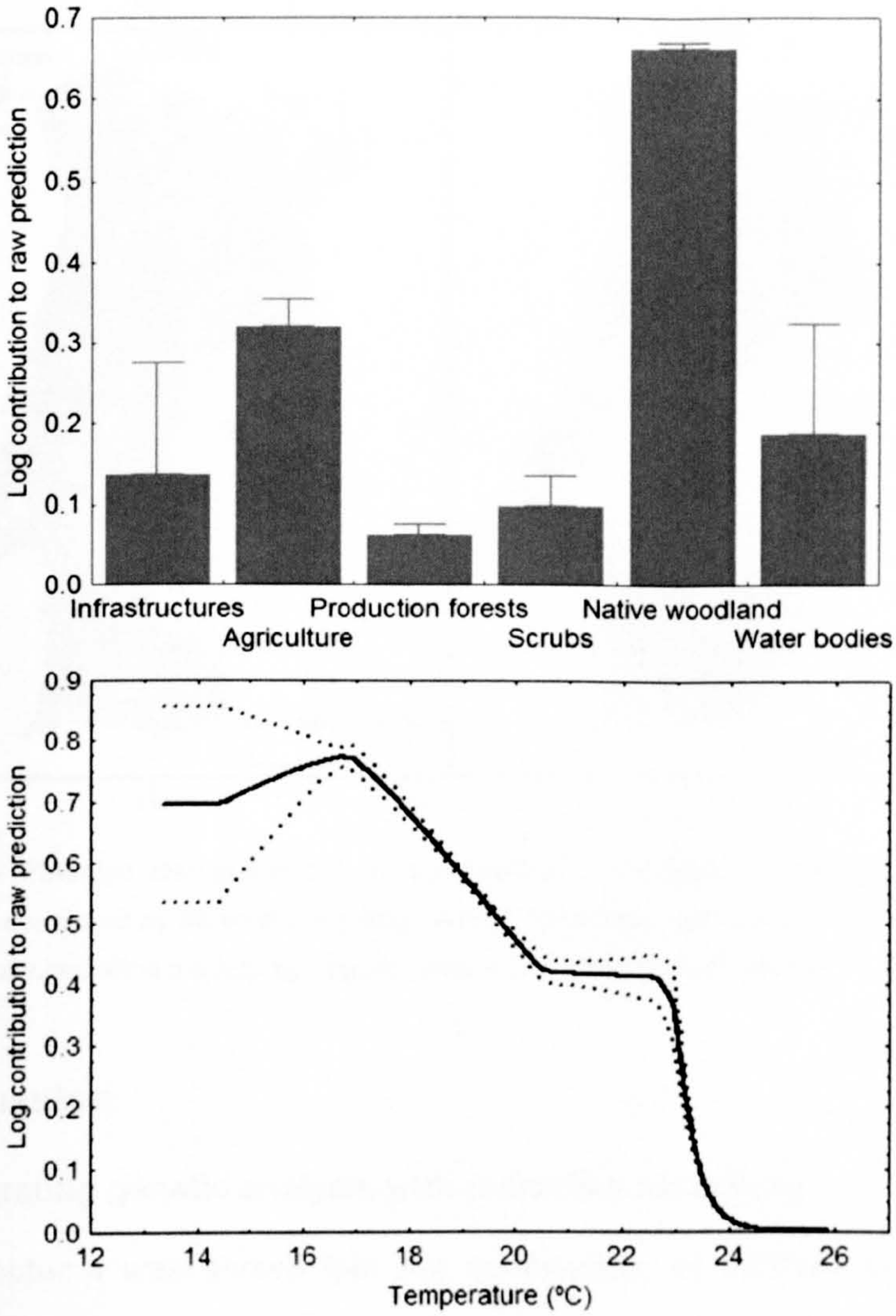

Figure 3.7 – Average response curves (with standard deviation) obtained in Maxent for the environmental factors related to *B. barbastellus* presence: (above) land cover and (below) average temperature of warmest quarter.

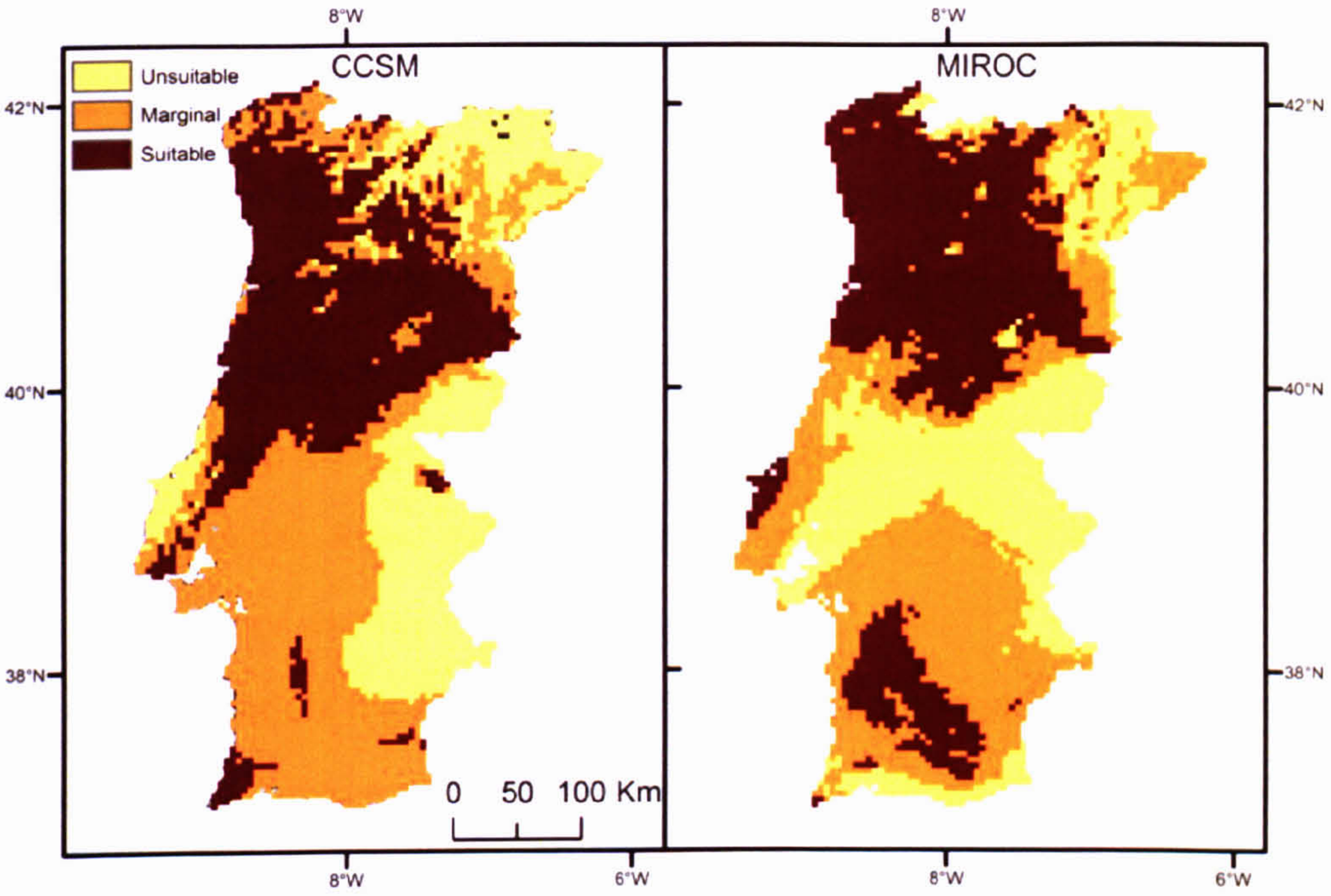

Figure 3.8 – Potential distribution for *B. barbastellus* in Portugal during the Last Glacial Maximum as computed by Maxent using only climatic variables. Left map was obtained with the CCSM general circulation model; right figure obtained with the MIROC model.

3.4 Discussion

3.4.1 Integrating genetic analysis with predictive modelling

In this chapter it was shown that the combination of mtDNA analysis with predictive modelling provided meaningful results for understanding conservation needs that might be undoubtedly more time and resource consuming by using more conventional methods (e.g. banding, acoustic transects, mist-netting, radiotracking). This is highly relevant when addressing conservation issues for rare species where knowledge gaps are generally wide, thus thwarting the development of adequate conservation management. It is of note that although more presence points were used in the model developed in this chapter (N=47) than in chapter two (N=17), the general pattern of the

predicted distribution for *B. barbastellus* in Portugal was very similar, despite a larger predicted area of occurrence in the south of the country predicted by the model used in the current chapter. Maxent is recognised to overfit predictions around presence records (Peterson *et al.* 2007), thus producing commission errors (i.e. the species occurs in areas predicted as unsuitable). By including more points, especially in the south where there existed no presence records previous to those found by my research summarised in chapter two, the commission error was reduced in that region.

Prior to this work, it might be expected that the fragmented distribution of the species could have influenced its population structure, resulting in a meta-population system where isolated populations are only connected by occasional migrants (Avise 2000). On the other hand, the flight ability and high dispersal potential of *B. barbastellus* could lead to an absence of deep population structure due to current high levels of gene flow. Additionally, habitat fragmentation and eventual isolation of populations may be too recent for genetic changes to be detected even if effective population sizes are low (Templeton *et al.* 1990; Bates 2002).

A number of unique haplotypes were found, especially in the northern and southern regions, thus suggesting some level of past isolation, or at least, low population connectivity. Moreover, unique haplotypes in the central region are singleton mutations, hence suggesting a recent drift from haplotypes also present in the northern populations. According to the LGM models the central region may have been a sink area where climatic conditions were too harsh to allow the fixation of populations. Indeed, it has been proposed that areas in the Iberian Peninsula may have functioned as "refugia within refugia" during glacial

periods (Gómez & Lunt 2007), where suitable areas were surrounded by vast unsuitable ones creating a complex meta-population system. The models predict that the suitable area in the north during the LGM was much wider than in the south. As a consequence, northern populations probably had a higher population size and density, hence after the end of the last glaciation they had a greater capacity to colonise new suitable areas and habitats, especially in the central region. The lack of shared haplotypes between southern and central population supports this hypothesis. This postglacial colonisation process probably occurred over a considerable time span. Mature broadleaf woodlands are the main foraging and roosting habitat for this bat (Russo *et al.* 2004; Hillen *et al.* 2009), hence the colonisation of new suitable climatic areas after the end of the glacial period may have been hampered by slow development of these forests in new areas (Garzón *et al.* 2007). Moreover, the slow rate of population dispersal could provide an explanation for the lack of detection of population expansion in all of the demographic statistics employed.

Furthermore, according the Φ_{ST} results none or at most low differentiation among the sampled populations was found, despite an overall high genetic diversity in *cyt b*. This suggests the existence of current gene flow despite the models for current conditions predicting the maintenance of a wide region of unsuitable conditions (although smaller than the predicted for the LGM) in the centre of the country. Therefore, results indicate that Portuguese *B. barbastellus* should be considered as a single management unit.

Only samples from Portugal were analysed, disregarding an eventual connection with populations from Spain. Nevertheless, known *B. barbastellus* distribution in Spain shows an even more fragmented pattern with the majority

of presence data located in the north and a few rather isolated records in the south suggesting a large area of unsuitable conditions in the centre of the Iberian Peninsula (Mitchell-Jones *et al.* 1999). As such, population structure in Portugal mirrors at a smaller scale what could be happening in the neighbouring country, yet population connectivity potential in Portugal between northern and southern populations might be higher. Bioclimatic conditions are more temperate and a milder climate exists along the Portuguese coastline whereas in Spain central regions climatic conditions (Sillero 2009) may be too harsh for the existence of *B. barbastellus* with temperatures higher than what they tolerate.

It can be argued that results could be limited by the exclusive use of mtDNA markers and its restricted maternal inheritance system. That said, the successful use of mtDNA markers in identifying population structure in bats still supports their applicability (e.g. Kerth *et al.* 2000; Newton *et al.* 2003; Ruedi & Castella 2003). Moreover, bat dispersal for the majority of temperate species is highly biased towards males (Petit & Mayer 1999; Ruedi *et al.* 2008) with females showing marked philopatry (Worthington Wilmer *et al* 1999; Kerth *et al.* 2002), reinforcing these markers usefulness for bat population studies.

Additionally, greater haplotype diversity was found in *cyt b* than in D-loop which was given that divergence rate of *cyt b* has been calculated at 4.7% Myr^{-1} for the bat genus *Myotis* (Ruedi & Mayer 2001) while the studied D-loop fragment is assumed to diverge at 20% Myr^{-1} (Petit *et al.* 1999). In addition, Salgueiro *et al.* (2007) also concluded that this D-loop fragment evolved 5.3 times faster than *cyt b* (based on HKY pairwise distances). These contrasting results may

be a consequence of asymmetric genetic drift or the lack of neutrality in, at least, parts of *cyt b*.

Land cover, specifically Portuguese native woodland was the most important ecological factor delimiting *B. barbastellus* distribution while the bats also avoided areas with high temperatures. The bioclimatic model showed that there is a large area of unsuitable climatic conditions in the south (where higher temperatures are recorded), although the distribution of *B. barbastellus* extends slightly further than suitable climatic conditions suggest. This might give a possible explanation why its occurrence is scarce in the south (almost restricted to the shoreline and mountain regions) in spite of the existence of extensive native woodland areas. In contrast, in the northern and central regions suitable land cover, and not climate, is probably the limiting factor for *B. barbastellus* existence because the suitable bioclimatic area is greater than its distribution predicted by the model with all variables included.

3.4.2 Implications for conservation

This chapter results have clear implications for the conservation of this rare species. Taking into account both genetic and predictive modelling results, conservation measures should focus on preserving Portuguese native mature woodland wherever *B. barbastellus* presence has been confirmed or predicted. This bat roosts inside dead trees or under bark, and hence only mature woodland has the potential to include suitable roosts (Russo *et al.* 2004). Moreover, the bats feed on insects inside forests (Greenaway 2001) covering great areas in a single night (Hillen *et al.* 2009; Kerth & Melber 2009), hence reinforcing the need to have large woodlands for this species' sustainability.

The increasing rate of habitat deterioration over the 20th century was probably a key factor leading to the fragmented pattern of native woodland seen today (Moreira & Russo 2007) and fires and the spreading of production forests (mainly *Eucalyptus* spp. and pine) still constitute a major threat to the loss of native woodland in Portugal. Moreover, predictive modelling also showed that currently there is a low potential for northern and central populations to be connected to southern ones. In order to minimise this situation, conservation efforts should again be directed to preserve current mature woodland, as well as areas of regenerating native forest. Moreover, due to the current trend of still promoting the expansion of production forests, it would be ideal if patches of native woodland could be developed within those production forests. In the forthcoming decades those areas could act like corridors between southern and northern populations and according to several climate change scenarios, it is likely that *B. barbastellus* populations will suffer from further pressures from the new climatic conditions (see Chapter 5). The existence of healthy populations in native woodland would be essential for the production of offspring and migrants to colonise new areas, thus increasing the odds for the species' survival.

Future work should focus on a deeper genetic analysis, using for example microsatellites, to estimate effective population sizes and the contribution of each sex to the species' dispersal. Although modelling results point to the existence of a wide gap between northern and southern populations in both LGM and current conditions, genetic analyses do not support the existence of two isolated populations, and hence two different management units. In conclusion, Portuguese barbastelles are best considered as a single

management unit from a genetic perspective, despite the existence on unsuitable conditions between the north and the south of the country.

3.5 Supplementary material

Haplotype	10	52	105	243	297	321	345	428	487	495	498	538	612	642	945	984	1012	1119
1	A	T	C	T	G	C	G	C	T	G	A	G	G	T	G	A	A	G
2	.	.	.	C	.	.	A	.	.	.	.	.	.	C	.	G	.	.
3	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.
4	.	.	.	C	.	.	A	.	.	.	G	.	.	C	.	.	.	.
5	.	.	T	.	A	.	.	.	.	A	.	.	.	.	A	.	G	A
6	G	.	.	.	.	.	.	A	G	.	.	.	.	.	A	.	.	.
7	G	.	.	C	.	.	A	.	.	.	G	.	.	C	.	.	.	.
8	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
9	.	.	.	C	.	.	A	.	.	.	.	A	.	C	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
11	G	C	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
12	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
13	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.

Table S3.1 – Variable nucleotide positions within the 1140 bp sequence of *cyt b* analysed in 30 bats.

CHAPTER 4

No barriers for postglacial colonisation of Europe by the barbastelle, *Barbastella barbastellus*: agreement between molecular data and past predictive modelling

Abstract

1. The barbastelle (*Barbastella barbastellus*) is a rare forest bat with a wide distribution in Europe, yet little is known about its phylogeography. Here I combine results from analysis of variability in two mtDNA fragments with species distribution modelling to determine glacial refugia and postglacial colonisation routes. Additionally, I investigated whether niche conservatism occurs in this species.

2. Maxent was the species distribution modelling technique used to determine the potential distribution of *B. barbastellus* for the present and for the Last Glacial Maximum (LGM) in Europe and north Africa. Additionally, two mitochondrial DNA fragments: cytochrome-*b* and D-loop were sequenced and analysed to support phylogeographic inferences.

3. Glacial refugia were identified in the three southern European peninsulas: Iberia, Italy and the Balkans. These latter two refugia played a major role in the postglacial colonisation process, with their populations expanding to England and central Europe, respectively. Palaeo-distribution models predicted that suitable climatic conditions existed in the inferred refugia during the LGM. Nevertheless, the overlap between the current and the LGM distributions was almost non-existent in Italy and in the Balkans, meaning that *B. barbastellus* populations were forced to shift range between glacial and interglacial periods, a process that probably caused some local extinctions. In contrast, Iberian populations showed a "refugia within refugium" pattern, with two unconnected areas containing stable populations (areas where populations subsisted during both glacial and interglacial phases). The match between LGM models and the

refugial areas determined by molecular analysis supported the hypothesis of niche conservatism in *B. barbastellus*.

4. *Synthesis and applications*: I argue that geographic patterns of genetic structuring, together with the modelling results, indicate the existence of four management units for conservation: Morocco, Iberia, Italy and U.K, and the Balkans and central Europe. In addition, all countries sampled possessed unique gene pools, thus stressing the need for the conservation of local populations.

4.1 Introduction

The Earth's climate cooled considerably around 2.6 million years ago, giving start to the Quaternary period. Gradually glaciations began to dominate the climate, especially in the temperate zones, interrupted by shorter and warmer interglacial periods (Bintanja & van de Wal 2008). This cycle of expansion and retraction of the ice sheets repeatedly forced massive range shifts in many animal and plant species. With the advance of the ice sheet in the temperate zone, several species became confined to regions where ecological conditions permitted their survival – glacial refugia (Taberlet *et al.* 1998; Hewitt 1999). The formation of isolated populations in refugia led to the evolution of unique gene pools resulting from phenomena such as genetic drift and local adaptation (Hewitt 2000). Periods of isolation associated with glacial periods may lead to genetic differentiation among populations, while dispersal during interglacial periods may result in population connectivity, thus promoting gene flow (Hewitt 1999). The "genetic signatures" of these population movements are still present in current patterns of phylogeographic structure and levels of genetic variation (Avise 2000).

In Europe, glacial periods had profound effects on genetic and population structure in a number of species (see Weiss & Ferrand 2008). Three major glacial refugia in southern Europe have been proposed for the majority of terrestrial animals (Hewitt 2000) – the Iberian and Italian peninsulas and the Balkans. However, it should not be assumed that each of these regions was uniformly covered by areas suitable for the species. The eventual existence of fragmented distributions within refugia, together with the existence of gene flow resulting from range expansion in the interglacial periods, has resulted in

complex patterns of population genetic structure that still persist (Gómez & Lunt 2007; Grill *et al.* 2009).

By investigating the macro-geographical genetic structure of populations it is possible to reconstruct population histories and identify genetically distinct populations that could constitute relevant units for conservation (Kerth *et al.* 2008). The analysis of mitochondrial DNA (mtDNA) has been the primary tool in phylogeographic studies, due to the fast mutation rate of mitochondrial genes and because maternal inheritance makes it possible to determine where a species was able to establish populations (Avise 2000). Although very powerful, this technique does not allow the determination of spatial population boundaries during these past events.

Predicting past, present or future species distribution is a major challenge in ecology. With more information currently available on past climatic conditions (e.g. Waltari *et al.* 2007) together with the development of powerful distribution modelling techniques, it is now possible to predict the location of the glacial refugia and respective population boundaries (e.g. Hugall *et al.* 2002; Moussalli *et al.* 2009). Unfortunately, to my best knowledge no wide-scale land cover data are so far available with a relevant resolution (usually higher than 2.8°) that can be used to reconstruct habitat conditions in the distant past. Therefore, most past predictive modelling is based on climatic data only, and hence models only produce bioclimatic envelopes for a species. Besides, by projecting climatic envelopes generated from current climatic conditions to the past it is assumed that the species' climatic niche is constant over time (Peterson *et al.* 1999). Although it has been proposed that a number of animal and plant species have retained niche characteristics over time (Peterson *et al.*

1999), there has been a considerable debate on this issue (see Losos 2008; Wiens 2008). Under the theory of niche conservatism, when local ecological conditions change dramatically either the species moves to new suitable locations or extinction is probable (Wiens & Graham 2005). Testing the niche conservatism hypothesis would be possible by comparing results from palaeo-distribution models with genetic analysis (Knowles *et al.* 2007; Waltari *et al.* 2007; Cordellier & Pfenninger 2009; Moussali *et al.* 2009). If both of these methods identify similar glacial refugia then the existence of niche conservatism is supported (Peterson *et al.* 1999).

In this work, the phylogeography of the barbastelle, *Barbastella barbastellus* (Schreber, 1774) was studied over most of its geographical range. The barbastelle is one of the rarest European bat species with a suspected population decline due to reduction in the extent of occurrence (listed as "Vulnerable" A2C in IUCN Red List category; Vié *et al.* 2009). Consequently, it has a highly fragmented distribution over a widespread range that covers most of continental Europe and extends to northern Morocco (Urbańczyk 1999). It seems to be dependent upon native mature woodland, and hence deforestation and habitat fragmentation are probably associated with its suspected population decline (Russo *et al.* 2004). Due to this strong association with mature deciduous forest, it is expected that the range expansion and contraction of this species should broadly mirror the spatial location of this habitat, as well as of other species highly dependent on these forests. For European bats, the Balkans and Iberia seemed to be the main sources of postglacial colonisation, while Italy made a smaller contribution (Petit *et al.* 1999; Ruedi & Castella 2003; Rossiter *et al.* 2007; Kerth *et al.* 2008; Flanders

et al. 2009). The majority of phylogeographic studies showed that bats had a less evident population structure than similarly sized non-volant mammals (Kerth *et al.* 2008), and the lack of structure is more pronounced in migratory species (Petit *et al.* 1999; Worthington Wilmer *et al.* 1999; Russell *et al.* 2007). The ability of bats to fly gives them a higher potential to overcome geographical obstacles, such as rivers or mountains, hence promoting gene flow. There is little information on migratory behaviour in *B. barbastellus*, although several authors suggest that seasonal migrations may occur, covering distances up to 290 km (Rydell & Bogdanowicz 1997; Riede 2001).

Juste *et al.* (2003) made the first assessment on the phylogeography of *B. barbastellus* in Europe and concluded that a shallow population structure existed with low divergence among European populations (between 0.7-2.1% in mtDNA cytochrome *b*). Nevertheless, the limited number of samples analysed and the restricted geographical range covered (e.g. excluding samples from the typical European glacial refugia) in that study did not allow any conclusions to be reached on postglacial colonisation routes or even on the determination of population structure and demographic history of the species in northern and central Europe.

In this study, I combined ecological niche modelling with mtDNA analysis to assess the population history of *B. barbastellus* in Europe, and to locate glacial refugia and postglacial colonisation routes. Additionally, by comparing the predictions of distribution models with patterns of phylogeographic structure, I evaluated whether niche conservatism occurs in this bat. To my knowledge this theory has never been tested in bats. The first goal was to determine population structure and glacial refugia over the entire geographical range of

the species by employing analyses of two mtDNA genes: the complete cytochrome *b* and a fragment of the D-loop. Moreover, from this analysis I determined if populations contracted or expanded from the glacial refugia to the current interglacial areas of occurrence, or remained stable in the former. In a second step, ecological niche modelling was employed to determine current and past distributions, as well as to determine in which areas populations might persist through both glacial and interglacial periods. This latter analysis also allowed investigation of which climatic variables had the greatest influence in delimiting the distribution of *B. barbastellus*. Finally, by combining genetic and modelling results it was possible to identify management units which may have implications for the conservation of this threatened bat.

4.2 Methods

4.2.1 Study area and sampling

The study area encompassed all mainland Europe (west of Caucasus), the U.K., Ireland, all major Mediterranean islands and part of north Africa, extending between coordinates 71°31'N – 33°30'N and 10°45'W – 45°33'E. Thus, the study area included almost the entire geographical range known for this species (Fig. 4.1), except the Canary Islands. The eastern limits of the species range are still poorly known but are probably located in the Caucasus region, where *B. barbastellus* is likely to occur in sympatry with its sister species *B. leucomelas* (Horáček *et al.* 2000). Material suitable for DNA analysis (wing membranes tissue and hairs) was obtained from several international researchers in order to cover as much as possible of the species' range.

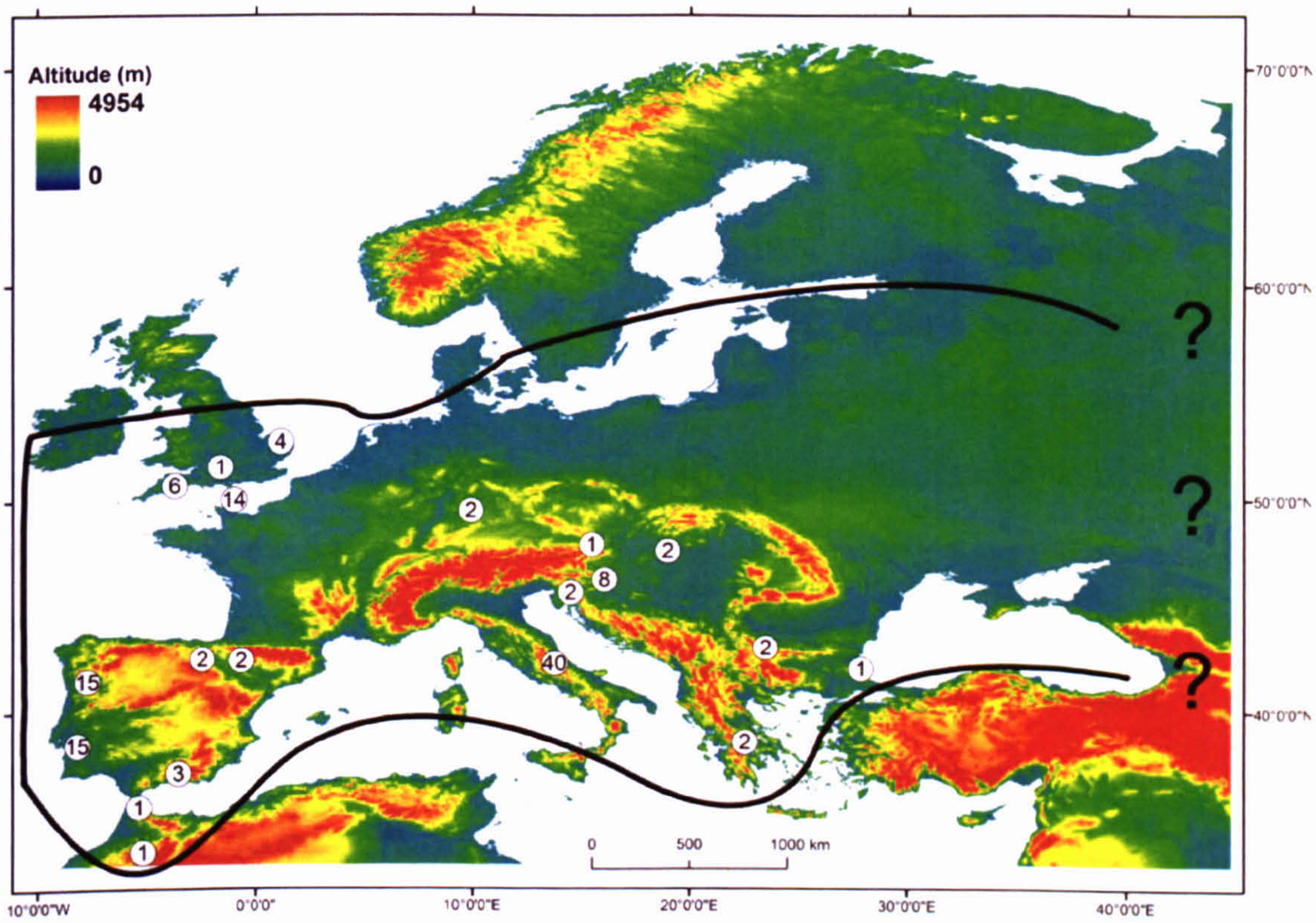

Figure 4.1 – Map of the approximate distribution of *B. barbastellus* in the western Palaearctic (excluding the Canary Islands) adapted from Urbańczyk (1999). Circles indicate the localities of the samples used in this study, while numbers within specify the sample size. Some circles may contain more than one sampled locality.

4.2.2 Sequence data

Genomic DNA was extracted from hair or wing punches using an E.Z.N.A. Tissue DNA kit (Omega Bio-Tek, GA, USA), eluted and stored in 50 µl of the provided elution buffer. Two mitochondrial genes were amplified: the cytochrome-*b* (*cyt b*) and the hypervariable domain (HVII) of the D-loop. To amplify those genes it was used the following sets of primers: Bat_*cytb*_1 (Li *et al.* 2006) and Bat_*cytb*_2 (Zhang *et al.* 2007) for *cyt b* and L16517 (Fumagalli *et al.* 1996) and sH651 (Castella *et al.* 2001) for D-loop. The polymerase chain

reaction (PCR) for *cyt b* was performed in 25 µl reaction volumes containing 5 mM $MgCl_2$, 0.24 µM of each primer, 0.6 µM of each dNTP, 1 unit of Taq DNA polymerase (QIAGEN, CA, USA) and 5 – 10 ng of genomic DNA. The amplification consisted of an initial denaturation at 95°C for 5 min, followed by 39 cycles of 95 °C for 40 s, 52°C for 45 s, and 72°C for 80 s, with a final elongation step at 72°C for 10 min. The amplified fragments of *cyt b* were sequenced on an automated sequencer (ABI 310; Applied Biosystems) in both directions using the same primers. Some samples (especially from museum specimens) contained degraded DNA, and hence it was not possible to obtain complete sequences using only the aforementioned primers. For these samples, the following internal primers were developed to produce shorter fragments: 5'-ATCACCGCCCTATTAACCCTA-3' (CytbatF2B) and 5'-GGTTGTTTGACCCTGTTTCG-3' (Cytbat R1), 5'-TTTAAAGAAACATGAAACGTAGGG-3' (CytbatF1). The overlapping fragments of *cyt b* were then assembled to produce sequences of 1140 bp. Regarding D-loop PCR conditions, the procedure described by Castella *et al.* (2001) was followed. The amplified fragments were sequenced in one direction using primer L16517 producing a sequence of 297 bp. Sequences from both genes were examined, edited and aligned using the software Bioedit v.7.0.1 (Hall 1999). All sequences are to be submitted to GenBank.

4.2.3 Genetic diversity and phylogenetic analyses

The two analysed mtDNA fragments are located on the same locus, and hence they were concatenated into a single sequence for all subsequent analyses (Grill *et al.* 2009). To describe the diversity of DNA sequences, basic

descriptive statistics and genetic diversity parameters, namely haplotype diversity (*h*), nucleotide diversity (π), genetic diference (d) and characterization of polymorphic sites were calculated using the software DnaSP v5.00.04 (Librado & Rozas 2009). Genetic divergence between regions was computed by pairwise Φ_{ST} and by performing a global test of differentiation among samples.

For the phylogenetic analysis sequences were imported into PAUP* 4.0b10 (Swofford 2003). Only unique haplotypes within *B. barbastellus* were included, while sequences from the only other two species within the genus, *B. leucomelas* and *B. beijingensis*, were used as outgroups (downloaded from GenBank; accession numbers EF534762 and EF534765/6). To estimate evolutionary relationships I used the Neighbour-Joining (NJ) and Maximum Parsimony (MP), as well as Maximum Likelihood (ML) analysis with random sequence addition (10 replicate heuristic searches). Support for nodes was estimated using the bootstrap technique (Felsenstein 1985) with 1000 replicates. From this analysis, haplotypes were clustered into four groups for subsequent analysis, namely Morocco, Iberia, Italy and U.K, and Balkans and central Europe.

4.2.4 Population structure and phylogeographic analyses

In order to evaluate relationships among closely related haplotypes, sequences were joined in unrooted networks, constructed with a 95% parsimony criterion using TCS 2.1 (Clement *et al.* 2000). With this method it was intended to detect potential spatial patterns existing according to the distribution of haplotypes.

Moreover, this technique is particularly suited to the analysis of single species gene genealogies, where ancestral and descendant haplotypes may coexist.

To test for the geographical genetic structure, an analysis of molecular variance (AMOVA) with 10 000 permutations was used in Arlequin 3.1 (Excoffier *et al.* 2006). In addition, pairwise Φ_{ST} was calculated to assess the amount of haplotypic subdivision. To examine the demographic history of *B. barbastellus*, pairwise mismatch distributions were plotted and tested to a model of rapid expansion. This analysis compares the observed frequencies of pairwise nucleotide differences to those expected from a model under a single rapid demographic expansion (Rogers & Harpending 1992). One thousand parametric bootstraps were used to generate the expansion model, while the sum of square deviations (SSD) between the observed and predicted mismatch distributions was calculated in order to determine the proportion of simulations producing larger expected SSD values than observed. In addition, because population changes are expected to leave detectable patterns in the distribution of pairwise differences, the smoothness of this distribution was measured by calculating the raggedness index. Distributions were generated for three regions (Iberia, Italy and England, and Balkans and central Europe). Because a limited number of samples was obtained from Morocco these were not considered for the analyses of demographic history.

Additionally, to test the hypothesis of demographic expansion it was calculated the Fu's neutrality statistic *Fs*, which tests the probability of observing a random neutral sample with no more alleles than the observed value of pairwise differences in the sample (Fu 1997), and the Tajima's *D* which tests whether the parameter derived from the average number of pairwise nucleotide

differences is equal to the parameter derived from the number of segregating sites in the sample (Tajima 1989). The significance of these tests was calculated using 10 000 coalescent simulations.

4.2.5 Predictive modelling for the present and LGM

For model training all available locations of *B. barbastellus* in Europe (N=538) were used as the dependent variable. Presence data were obtained from Urbańczyk (1999) available from the European Environment Agency website (http://eunis.eea.europa.eu) which covered the majority of the known distribution. The reliability of the Irish presence record in this dataset is controversial (only a single echolocation recording), however this record had little weight on the predictive modelling due to the high number of presence records in the whole dataset. Furthermore, a set of independent ecogeographical variables (EGV) was also considered: annual average temperature (°C), annual average precipitation (mm), average temperature range (°C), average temperature of the warmest quarter (°C), average temperature of the coldest quarter (°C), average precipitation of the wettest quarter (mm) and average precipitation of the driest quarter (mm) (Hijmans *et al.* 2005; WorldClim dataset available at http://www.worldclim.org). Chosen climatic variables are acknowledged to exert a strong influence on bat distribution patterns (Ulrich *et al.* 2007), as they are associated with crucial aspects for their survival such as metabolic rate, gestation times and evaporative water loss (Racey *et al.* 1987; Webb *et al.* 1995; Adams & Hayes 2008).

A presence-only modelling technique was chosen because reliable absence data were not available and the elusive and nocturnal behaviour of bats adds even more uncertainty to absences. Species distribution modelling techniques have been extensively tested for different sample sizes, geographical ranges and resolutions. I used a maximum entropy modelling technique (Maxent species distribution modelling, v.3.3.0; http://www.cs.princeton.edu/~schapire/maxent), that estimates the range of a species by finding the maximum entropy distribution given the constraint that the expected value for each EGV closely matches the empirical average of the presence data (see Phillips *et al.* 2006). When compared with other modelling techniques, Maxent has achieved a very good performance on the statistical indices measuring accuracy on predicting a species' distribution (Brotons *et al.* 2004; Elith *et al.* 2006; Hernandez *et al.* 2006; Wisz *et al.* 2008). Models were run with 80% of the presence data while the remaining 20% were used to test them. Because Maxent randomly chooses which presence data to include in the training or test models, this would imply that models produced would be different according to the chosen presence data. Consequently, 100 model replications were ran and averaged into a single model (with standard deviation shown when appropriate). Calculations where done in the autofeatures mode with a maximum of 1000 interactions and regularization set to 0.5. Generated models had a continuous output ranging from 0 to 1, where 1 indicates that species presence is highly probable.

To verify which variables were the most important for model building, a Jackknife analysis of the gain (a measure of likelihood between the presence data and ecological variables) was made with the training data being the results

that are presented in graphics. The relationship between the species' presence and the most relevant EGVs was assessed by the analysis of response curve plots obtained with univariate models. The obtained model was then tested with Receiver Operated Characteristics (ROC) plots to evaluate their predictive ability. The area under the curve (AUC) of the ROC analysis provides a single measure of the model performance (Liu *et al.* 2005) and ranges from 0.5 (randomness) to 1 (perfect discrimination). An AUC score higher than 0.7 is considered to represent good model accuracy (Fielding & Bell 1997).

Regarding model production, bioclimatic models were calculated for the present and projected them to the Last Glacial Maximum (LGM; 23 000–18 000 years BP) using the above selected climatic variables. Two different General Circulation Models (GCM) were used for this latter period, the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC, version 3.2). For more information on the LGM climatic model development and downscaling see Hijmans *et al.* (2005) and Waltari *et al.* (2007). All digital information (variables and presence data) had a resolution of 0.5º (~55 km), thus the study area included a total of 6283 cells for the present conditions and 8050 cells for the LGM projections (cell numbers increased due to the advance of the coastline during the glacial period).

In order to determine in which areas populations were present during glacial and interglacial periods, it was also necessary to produce binary presence/absence maps for all models. The 10% of training presence was used as the threshold value, a value above which the species is assumed to be present (Suárez-Seoane *et al.* 2008; Lamb *et al.* 2008; Raes *et al.* 2009). This threshold takes into account that for large datasets collected by different

researchers over great time spans, some errors may occur regarding species identification or in the geographic referencing. After model reclassification it was then possible to determine which regions had suitable climatic conditions for *B. barbastellus* populations during both glacial and interglacial periods, thus areas with stable populations over the studied period (Moussalli *et al.* 2009). This was achieved by multiplying reclassified binary models.

4.3 Results

4.3.1 Sequence data and genetic diversity

A total of 115 samples were successfully amplified for the complete *cyt b* (1140bp) and a fragment of the D-loop (297bp). Sequences belonging to the same individual were aligned and concatenated into a single one with a total length of 1437bp. Forty-nine haplotypes were found (see Table S4.1, Supporting information) resulting from 69 variable sites of which 56 were transitions and 11 were transversions, and 44 sites were parsimony informative. The majority of the mutational sites (n=56) were located in the *cyt b* gene with only 13 variable sites found in the D-loop fragment. In addition, two indels were also found in the latter fragment. Considering the concatenated sequence, haplotype diversity was high (h=0.947±0.01) while nucleotide diversity was moderately low (π =0.007±0.0002). Overall mean genetic difference (p-distance) was low (d=0.007±0.002) which was in agreement with mean diversity within the four populations (d=0.003±0.001).

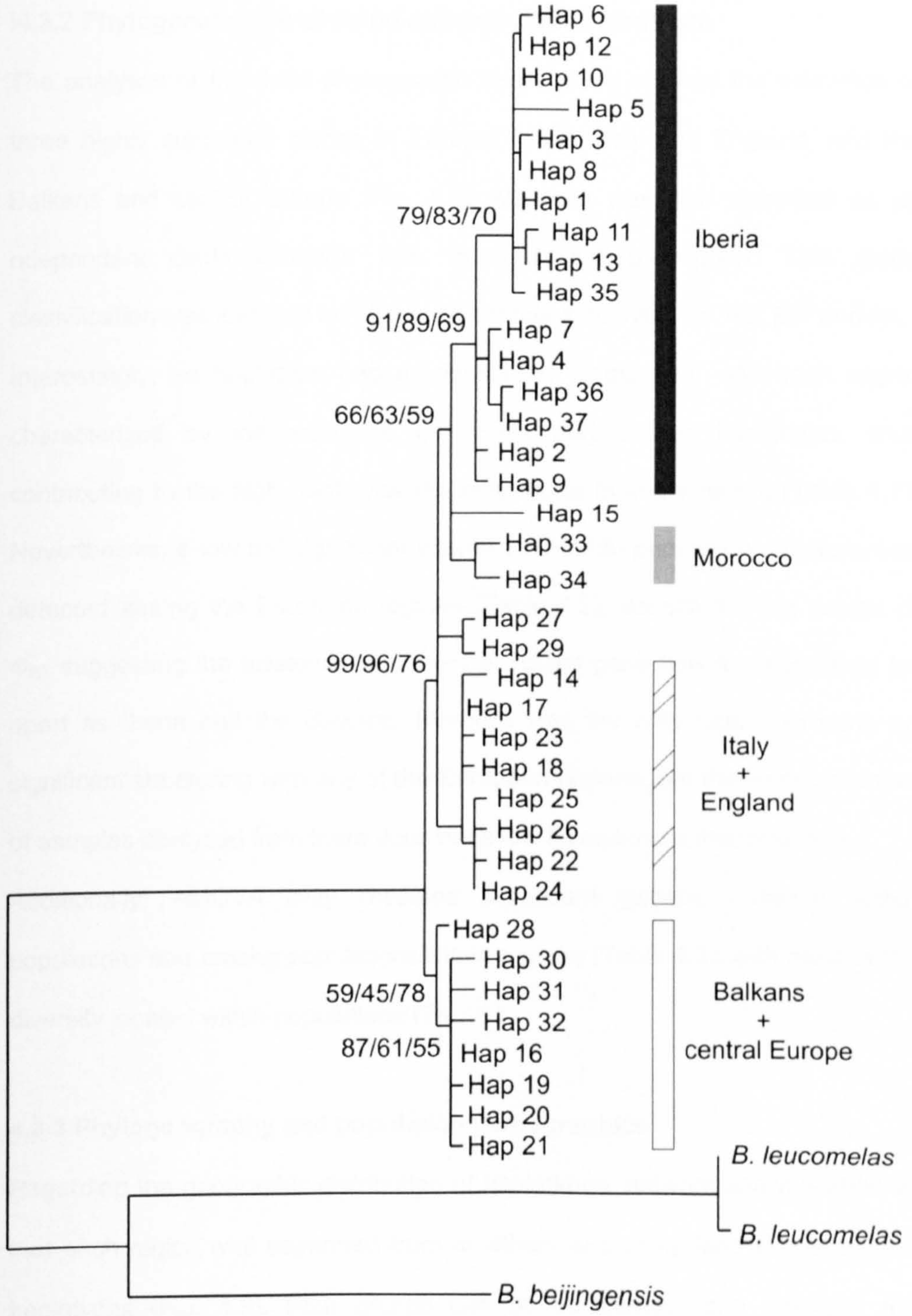

Figure 4.2 – Phylogenetic relationships between haplotypes as shown by the Neighbour-Joining consensus tree (other two methods produced trees with a similar structure). Bootstrap values are indicated for Neighbour-Joining, Maximum Parsimony and Maximum Likelihood.

4.3.2 Phylogenetic sub-division and population structure

The analyses of the three phylogenetic trees clearly showed the existence of three highly supported clades in Europe: Iberia, Italy and England, and the Balkans and central Europe (Fig. 4.2). Morocco was also classified as an ndependent clade although with lower bootstrap support. This clade classification was identical for all three methods employed, i.e. NJ, MP and ML. Interestingly, no haplotype had a widespread distribution, with each region characterized by the existence of almost only unique haplotypes, thus contributing to the high haplotype diversity found in every region (Table 4.1). Nevertheless, a low but significant (Φ_{ST}<0.2; P<0.05) population structure was detected among the European regions (Table 4.2), despite the low values of Φ_{ST} suggesting the existence of current or recent gene flow for regions as far apart as Iberia and the Balkans. Morocco was the only region showing no significant structuring with any of the European regions, but the limited number of samples analysed from there does not allow a conclusive interpretation. Additionally, AMOVA only revealed significant genetic variance within populations and among populations/within regions (Table 4.3), with most of the diversity located within populations (78.7%).

4.3.3 Phylogeography and population demographics

Regarding the geographic distribution of haplotypes, network analysis showed that each region was separated from all others and comprised almost unique haplotypes (Fig. 4.3). Four groups can be considered, one including the Moroccan samples alone, whose closest affinity seemed to be with the Iberian populations. In Iberia two populations seem to exist, one of which included haplotypes only present in northern Spain and Portugal. Those two populations

Group	Sample size	Number of haplotypes	Polymorphic sites	Nucleotide diversity	Haplotype diversity
Iberia	36	22	32	0.004	0.96
Italy+England	56	15	33	0.003	0.836
Balkans+central Europe	20	11	24	0.002	0.805
Morocco	2	2	4	-	-

Table 4.1 – Diversity statistics based on 1437 bp of *B. barbastellus* grouped in four regions, as determined by the analysis of the phylogenetic trees.

	Iberia	Italy+England	Balkans+central Europe	Morocco
Iberia		*	*	n.s.
Italy+England	0.104		*	n.s.
Balkans+central Europe	0.113	0.165		n.s.
Morocco	0.029	0.127	0.15	

Table 4.2 – Genetic differentiation of *B. barbastellus* (Φ_{ST}) among the defined four regions presented in the lower matrix (*P<0.05; probability that observed heterozygosity differed from expected).

Structure	Source of variation	Variation (%)	Fixation indices	P value
Four regions	Among regions	1.42	Φ_{CT} 0.014	0.43
	Among populations/within regions	20.25	Φ_{SC} 0.201	0.000
	Within populations	78.33	Φ_{ST} 0.217	0.000

Table 4.3 – Analysis of Molecular Variance (AMOVA) measured among populations of *B. barbastellus* for its entire range. The four regions correspond to Morocco, Iberia, Italy + England and Balkans + Central Europe as determined by the analysis of the phylogenetic trees.

seem to have expanded only within Iberia and apparently none succeed in passing the Pyrenees towards central Europe, apart from the presence of a Spanish haplotype (47) closely related to the British ones (which could result from current gene flow). Britain seems to have been colonised mainly by batsthat originated from Italy. There were possibly a few events that led to bats

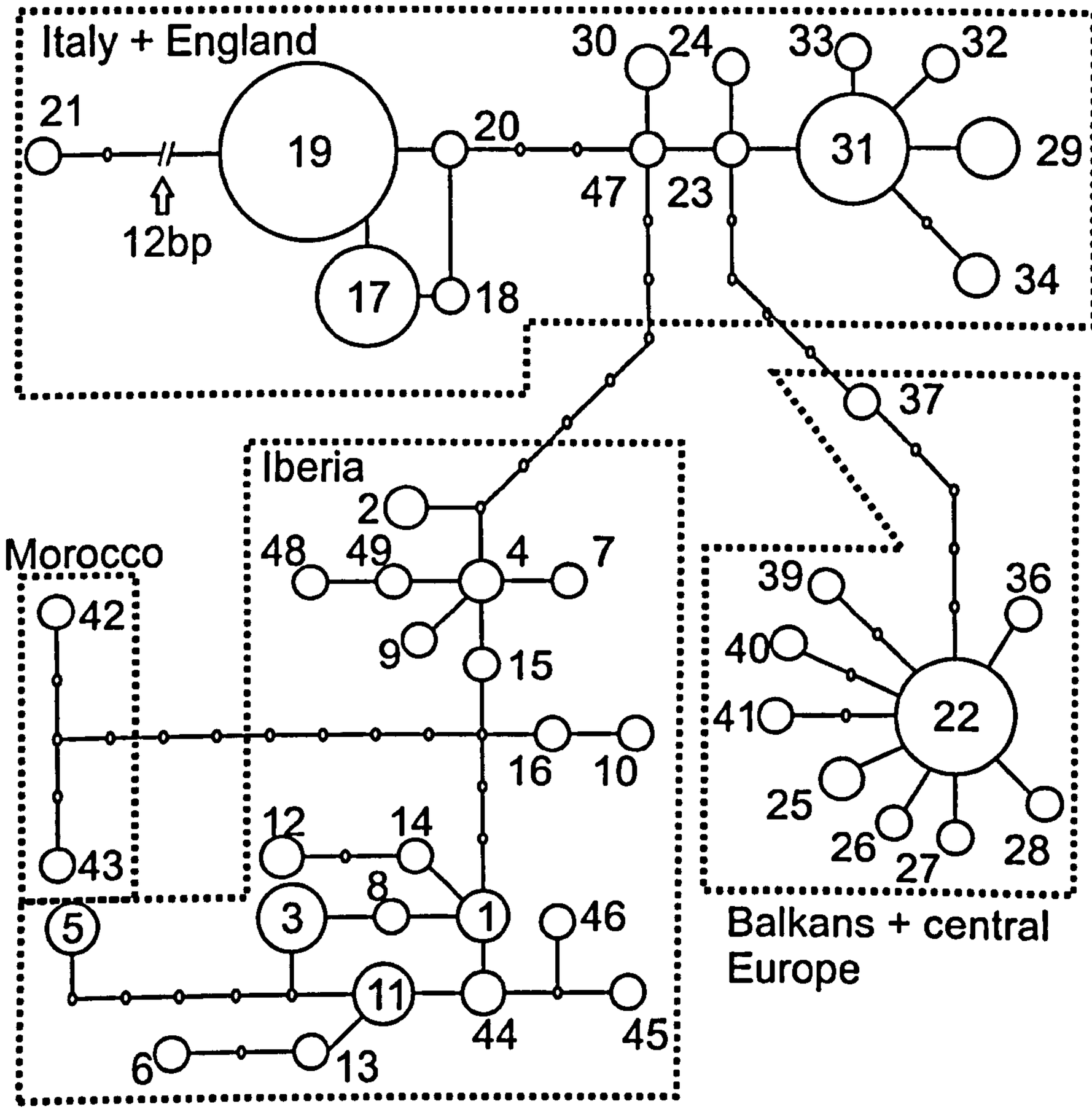

Figure 4.3 – Concatenated (cytochrome *b* and D-loop) minimum spanning network based 1437 bp for 115 barbastelle specimens. Clades are delimited by the dashed boxes. The dots connecting the network represent missing or unsampled haplotypes. Circles represent haplotypes, and their size is proportional to the number of specimens; haplotypes are designated by numbers that correspond to Table S4.1, in Supplementary material.

colonising the British mainland, although the majority of the haplotypes present resulted from a population expansion of haplotypes currently represented by those from the Isle of Wight in the English Channel (haplotype 31). The fourth group mainly comprised Balkan haplotypes and showed a clear star-structure, indicating an expansion from this region to other parts of the Balkans and

Statistics	Iberia	Italy	Balkans
SSD	0.009	0.026	0.011
P_{SSD}	0.444	0.349	0.434
Raggedness index	0.019	0.043	0.037
$P_{raggedness}$	0.560	0.479	0.880

Table 4.4 – Demographic statistics of *B. barbastellus* grouped in three regions Iberia, Italy + England and Balkans + Central Europe as determined by the analysis of the phylogenetic trees.

central Europe (in this study represented by Germany and Hungary). The dominant haplotype (22) was present in the Balkans and central Europe, but also in England, suggesting the existence of secondary colonisation events into Britain. None of the regions studied achieved a significant *P* value for the mismatch distribution scenario of rapid demographic expansion (Table 4.4), which is in agreement with the raggedness results. Moreover, the mode of the mismatch distribution was shifted towards higher values, especially in Iberia, that together with the multiple peaks observed in the number of pairwise differences, suggests that populations have remained of constant size (Fig. 4.4). In contrast, the more sensitive Fu's *Fs* test and Tajima's *D* showed that in Iberia (*Fs*=-8.11, *P*<0.005; *D*=-2.56, *P*<0.0003) and the Balkans (*Fs*=-4.85, *P*<0.04; *D*=-2.44, *P*<0.0001) a scenario of population expansion is supported, while in Italy (*Fs*=-1.13, *P*<0.36; *D*=-1.30, *P*<0.11) the non-significant results indicated no expansion. Moreover, the negative values obtained in the Fu's *Fs* (ranging from -8.11 to -1.13) statistic suggest that there was an excess of recent mutations or rare alleles all over Europe.

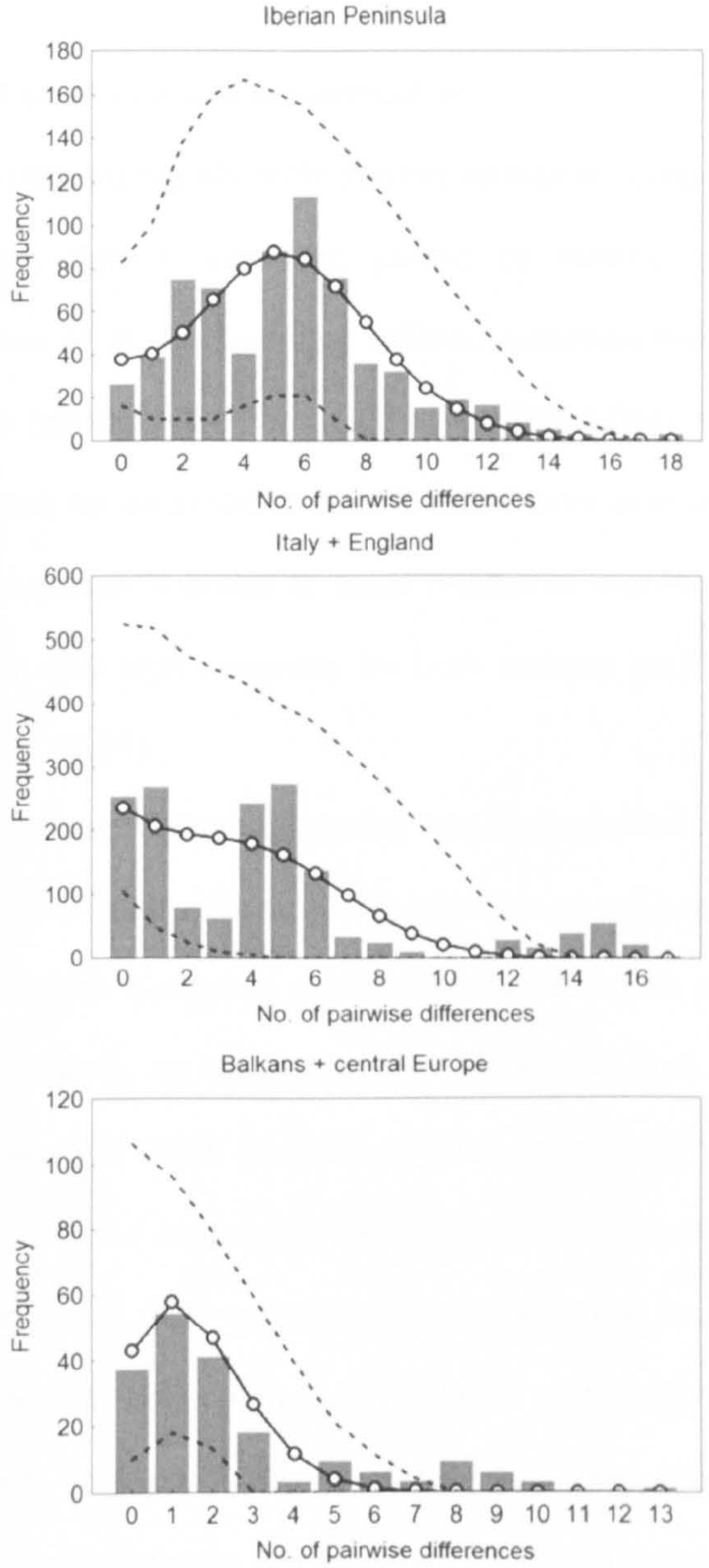

Figure 4.4 – Mismatch distributions for three regions, Iberia, Italy and England, and the Balkans and central Europe. The grey bars show the observed distribution of pairwise nucleotide differences. The solid line represents the expected distribution under a model of sudden demographic expansion, while dashed line indicate the lower and higher 95% confidence intervals.

4.3.4 Current and LGM predicted distribution

Model predictions differed significantly among all model comparisons ($P<0.001$, two-tailed Wilcoxon signed rank test, paired by model), showing that the potential distribution of *B. barbastellus* differed between the present and the LGM, as well as between the two GCMs for the LGM. Even so, climatic conditions presented for each GCM were much colder and drier than present. However, this divergence seemed to have negligible impact on model quality as predictions had very high accuracy for both training (AUC=0.92±0.01) and test data (AUC=0.87±0.01).

The model for present conditions predicted that *B. barbastellus* distribution was mainly concentrated in central Europe, although the species was also predicted to occur in the southern European peninsulas, in the south of England and in the south of Scandinavia, as well as in a considerable area in the Caucasus (Fig. 4.5). Regarding the LGM distribution, both models almost restricted the species' occurrence to the southern European peninsulas, although each GCM differed in the extent of the predictions. Moreover, the locations where the species could potentially occur during both glacial and interglacial stages were severely delimited by the smaller extent of the LGM distributions. Nevertheless, it was surprising to predict only small areas of occupancy during the present and during the LGM in both Italy and the Balkans, suggesting that bats were forced to move to different areas within refugia between interglacial and glacial periods. On the other hand, a considerable area of predicted overlap between current and LGM distributions was found in Iberia, with two unconnected areas of potential occurrence, one extending form the north (Pyrenees) to the west

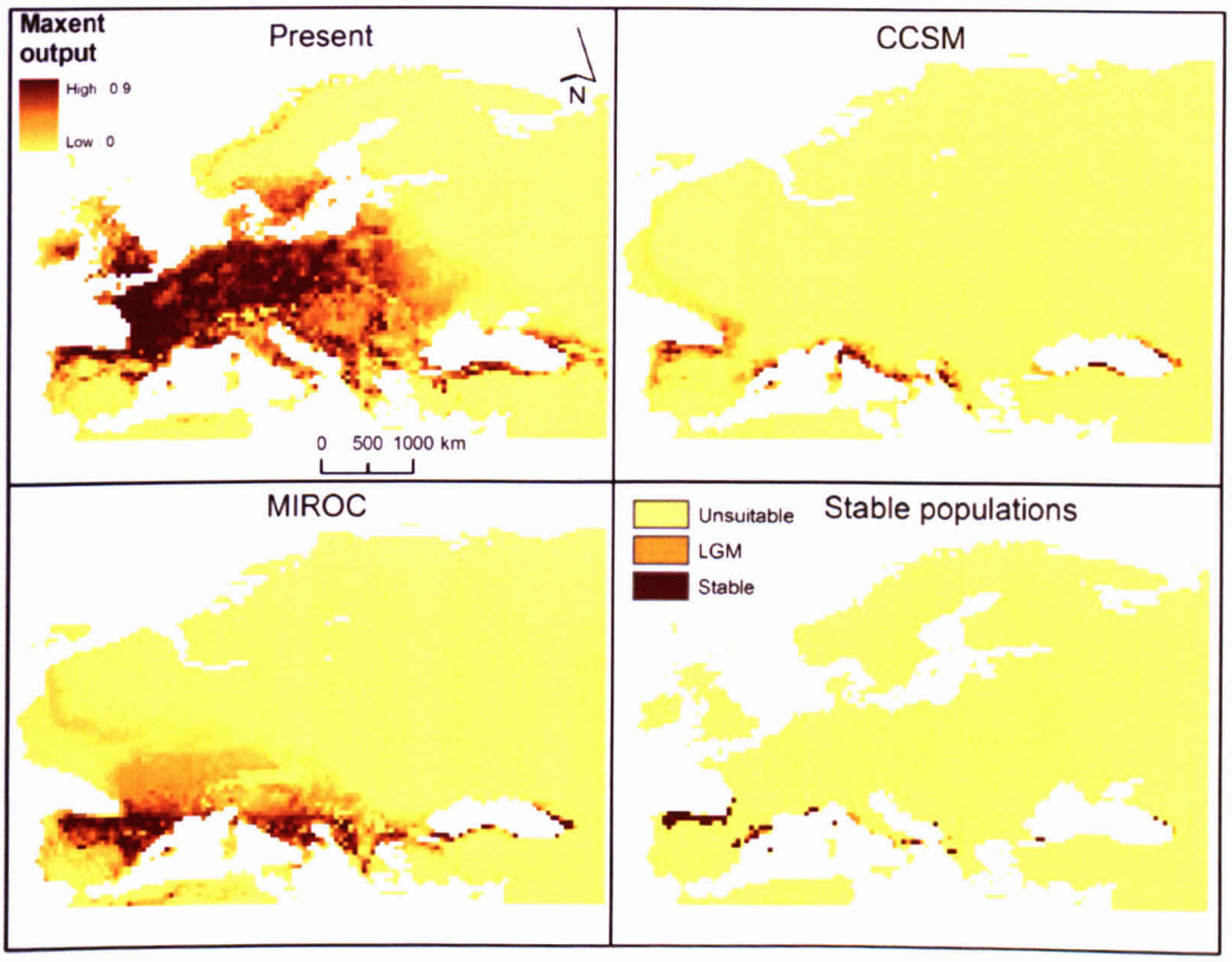

Figure 4.5 – Four Maxent models of the potential distribution of *B. barbastellus*. One for the present conditions; two for the Last Glacial Maximum employing two different general circulation models (CCSM and MIROC); and a reclassified map indicating areas where suitable conditions subsisted in the LGM alone and in both glacial and interglacial periods (here called stable).

(north of Portugal) and another in the southeast (mainly around the Ebro basin). Also of note, the eastern limit of the species' distribution was predicted to occur in the Caucasus during both glacial and postglacial periods.

4.3.5 Relevant ecogeographical variables

Average temperature of the coldest quarter was clearly the most important variable, the one with greatest contribution and most uncorrelated information

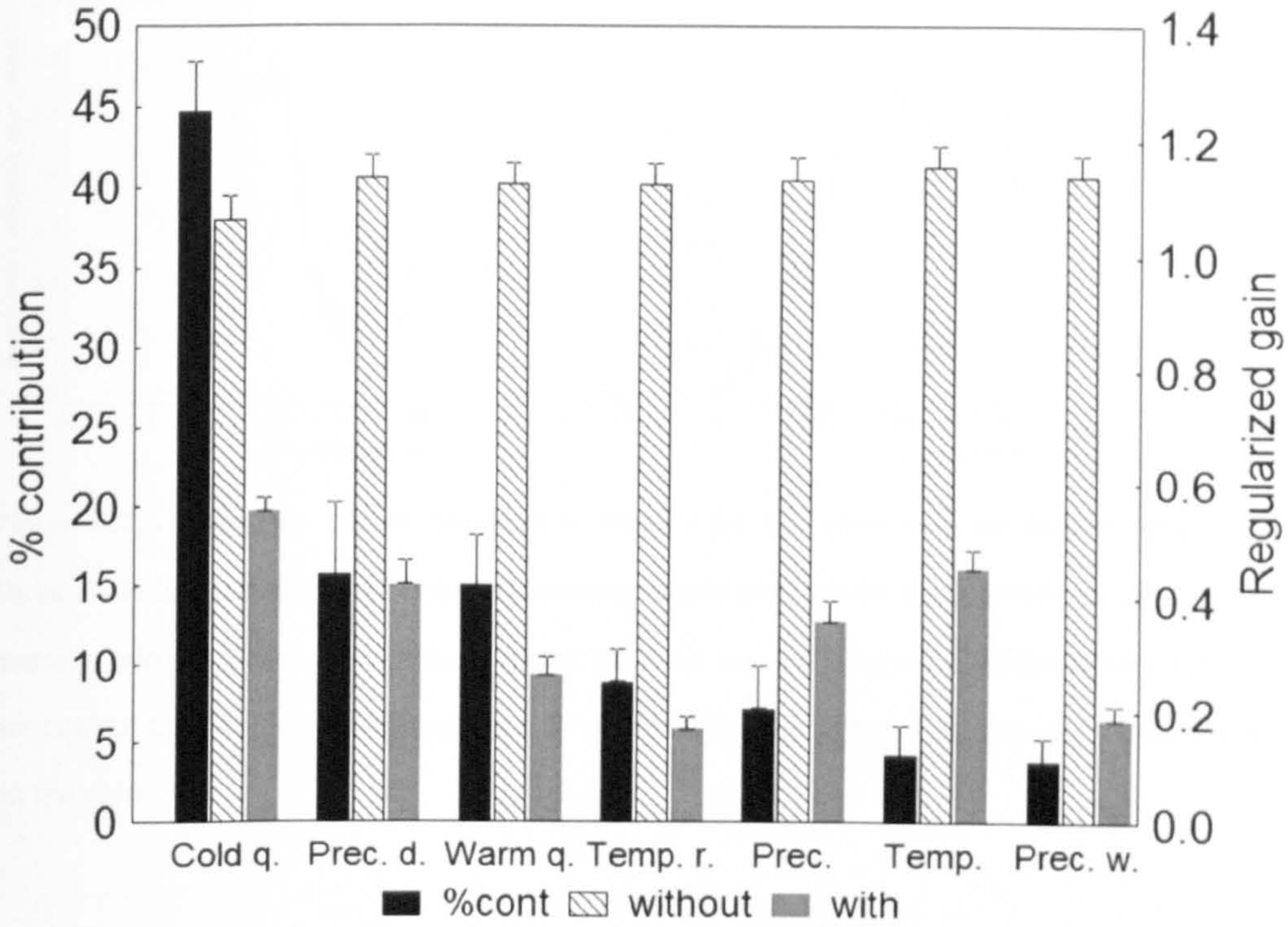

Figure 4.6 – Representation of each variable's importance in the Maxent model. The percentage of contribution of each variable to the model is represented by the black bar and corresponding values may be found on the left axis. The other two bars represent the jackknife results for the model with only one variable ("with") or with all variables but the analysed one ("without"). Values for these results are represented in the right axis. Cold q., average temperature of the coldest quarter (ºC); Prec. d., average precipitation of the driest quarter (mm); Warm q., average temperature of the warmest quarter (ºC); Temp. r., average temperature range (ºC); Prec., annual average precipitation (mm); Temp., annual average temperature (ºC); Prec. w., average precipitation of the wettest quarter (mm).

for the model (Fig. 4.6). Precipitation in the driest quarter and average temperature of the warmest quarter also showed some relevance. The range of variable values where *B. barbastellus* occurs suggests a preference for milder climates, and avoidance of areas with extreme temperatures and extreme precipitation values (Fig. 4.7).

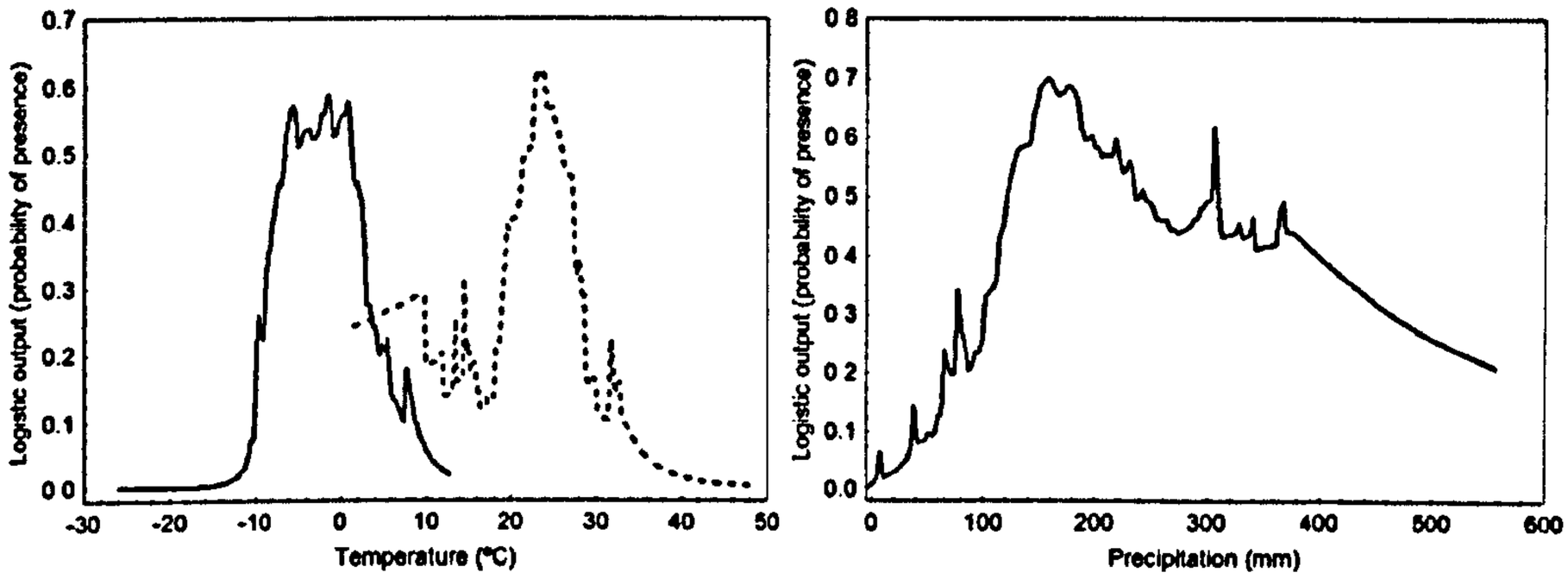

Figure 4.7 - Response curves obtained in Maxent for the environmental factors related to *Barbastella barbastellus* presence: temperature values (left) where the dashed line represents average temperature of the warmest quarter (°C) and the solid line the average temperature of the coldest quarter (°C); the precipitation of the driest quarter (mm) is represented in the graph on the right.

4.4 Discussion

4.4.1 Refugia localization and postglacial colonisation in *B. barbastellus*

To my knowledge, this study is the first employing both past predictive modelling and analysis of molecular data to determine the spatial distribution of glacial refugia for bat populations. Both techniques were in agreement, indicating that the southern European peninsulas, Iberia, Italy and the Balkans, acted as the main glacial refugia for *B. barbastellus* in Europe. After the end of the glacial period, when suitable conditions emerged elsewhere, colonisation of northern territories occurred mainly from the Balkans, with bats spreading into central Europe, and from Italy, with movement northwest into England. These results suggest that there were no barriers to the dispersal of *B. barbastellus*, and bats could even disperse over mountain chains such as the Alps (Aellen 1983). As such, the lack of importance that Iberia had on patterns of postglacial colonisation was surprising. According to both of the models and the genetic

results, some populations may have persisted near the Pyrenees, hence in an advantageous location for colonising Britain. In fact, one Spanish haplotype was closely related to one found in Britain, although this may reflect secondary waves of colonisation. Only by thorough analyses of the French populations (not included in this study) can we eventually clarify this point. Another interesting result points to the species' occurrence in the Caucasus during both glacial and postglacial periods. These *B. barbastellus* populations probably persisted when isolated from the other European populations, while overlapping with the westernmost range of its sister species *B. leucomelas* (Horáček *et al.* 2000). It would be interesting to analyse samples from the Caucasus, in order to clarify if those populations belong to a unique evolutionary lineage within *B. barbastellus*, or even if hybridisation with *B. leucomelas* occurs.

Postglacial colonisation was probably limited by the slow development of suitable habitat in the north. Mature woodland is the main foraging and roosting habitat for this bat (Russo *et al.* 2004; Hillen *et al.* 2009), and yet the development of these forests is slow, taking at least several decades (McLachlan *et al.* 2005). The mismatch analysis results rejected the existence of a sudden population expansion, while the more sensitive Fu's *Fs* detected population expansion in Iberia and Balkans. The situation in Italy is less clear. Despite all statistics rejecting evidence of a population expansion, the fact that only one region was sampled within Italy (where the bat has a very restricted current distribution) may have influenced this result.

Comparing the phylogeography of *B. barbastellus* with that of other European bats, there are considerable differences in the proposed glacial refugia and the

suggested routes of postglacial expansion. For *Myotis myotis*, central Europe was mainly colonised by Iberian populations (Ruedi & Castella 2003), while for *Nyctalus noctula* and *Myotis bechsteinii* postglacial colonisation occurred from the Balkans (Petit *et al.* 1999; Kerth *et al.* 2008); the situation for *Rhinolophus ferrumequinum* is less clear with results pointing to the existence of at least two major glacial refugia (Rossiter *et al.* 2007; Flanders *et al.* 2009), the Balkans and another undefined western refugium (Italy and/or Iberia). Instead, phylogeographic patterns for *B. barbastellus* resemble the *hedgehog* colonisation paradigm proposed by Hewitt (1999; 2000), where the three southern European peninsulas contributed to postglacial range expansion, though in this study Iberia had little or no contribution for northern and central Europe. The *hedgehog* pattern is also matched by phylogeographic inferences about European oaks', where the three southern European glacial refugia are recognized to be the source of northern European populations (Ferris *et al.* 1998). This may give a possible explanation of why *B. barbastellus* exhibited this pattern because broadleaf woodland is recognized to be the main foraging and roosting habitat for this bat (Russo *et al.* 2004; Hillen *et al.* 2009).

A number of missing haplotypes were inferred for the Central European group and especially in Italy where I found a difference of up to 13 mutations among haplotypes. These situations may result from incomplete geographical sampling (Cassens *et al.* 2003), and that all the Italian samples originated from a single region supports this hypothesis. Alternatively, missing haplotypes may now be extinct. According to LGM distribution models, Italian and Balkan populations were forced to shift their ranges between the glacial and interglacial periods. These were probably stressful periods for the populations due to the rapid

climatic changes, especially in the transition between the glacial to the interglacial period (Bitanja & van de Wal 2008). Bat populations in these regions could have been under pressure by occupying forested areas with unsuitable climatic conditions. Additionally, foraging habitats and suitable roosts could have been rare in areas of suitable climate, thus increasing the probability of extinction for some populations. The cycle of extinctions and founder effects may explain the absence of certain haplotypes in the network analyses, although only with more sampling in those areas could this question be fully addressed. In contrast, Iberian populations seem to have remained rather stable over time. The haplotype network shows a complex system with several homoplasies existing. However, in agreement with the palaeo-predictive modelling two groups can be considered in Iberia, one extending west from the Pyrenees to the north of Portugal and the other mainly located in the Ebro basin. The high genetic diversity found in Iberia could also be a consequence of genetic drift in these isolated refugia within the peninsula. The existence of gene flow during interglacial periods altogether with the isolation and extinction of some Iberian populations (and haplotypes) in the glacial phases could explain this intricate pattern.

4.4.2 Niche conservatism and climatic tolerance in *B. barbastellus*

The congruence in the location of glacial refugia in both genetic analyses and past predictive modelling suggests the existence of niche conservatism in *B. barbastellus*, at least through the late Pleistocene (Waltari *et al.* 2007). Furthermore, fossil records for the late Pleistocene are largely in agreement with the models (Rydell & Bogdanowicz 1997), with a match between the

location of model's high suitability values and the areas where *B. barbastellus* fossils have been found. Examples of that agreement are the existence of late Pleistocene fossils in southern Spain (Sevilla 1987) and Sicily (Kotsakis & Petronio 1980) where the species is now absent. Hence the species was distributed in the past in areas where the range of climatic conditions overlapped (at least partially) with current ones (Peterson *et al.* 1999). To my knowledge this is the first time that the existence of niche conservatism has been demonstrated in bats, adding this species to a number of animal and plant species where the absence of niche evolution has been demonstrated (Peterson *et al.* 1999; Martínez-Meyer *et al.* 2004; Cordellier & Pfenninger 2009; Dépraz *et al.* 2009). This has clear implications for conservation because results show that the species limits its dispersal to a particular climatic regime (Wiens & Graham 2005). Therefore, when ecological conditions change to those outside of the species' ecological tolerance, its populations will have to shift locations or extinction will become probable (Parmesan & Yohe 2003). Moreover, the congruence between model predictions and phylogeographic analyses strengthens the reliability of future climate change predictions (Wiens & Graham 2005).

It can be argued whether using climatic variables alone in predictive modelling accurately predicts a species' distribution, because biotic interactions so relevant for species survival such as competition, predation or suitable habitat availability are not considered. Nevertheless, at a continental scale such as the one employed in this study, abiotic variables seem to delimit large-scale distributions with better accuracy than biotic variables (Pearson & Dawson 2003).

Despite the predicted current distribution showed a large area of suitable conditions without many discontinuities, in reality the distribution of *B. barbastellus* is rather different, with populations showing a very fragmented distribution (Urbańczyk 1999). Fragmentation may not be detected by the models because the resolution of ca. 55 km employed does not allow us to draw conclusions at a local scale, meaning that within a pixel of predicted occurrence the bat may not exist all over its extent (Pearson & Dawson 2003).

The extreme temperature values (minimum and maximum), together with water availability in the summer were the most relevant factors in delimiting the geographic range of *B. barbastellus*. These variables are acknowledged to have great relevance for explaining the geographical ranges of European bat species (Ulrich *et al.* 2007) and also have a great influence on bat physiology. Climatic characteristics can affect the survival of bats because their physiology is adversely affected when temperatures lay outside optimum conditions (Racey *et al.* 1987; Webb *et al.* 1995; Adams & Hayes 2008). Furthermore, insects, or more specifically moths, are the main prey of *B. barbastellus* (Sierro 1999) and are also affected by climatic conditions. Areas with extreme temperatures and/or dry climate will probably have a lower abundance and diversity of insects (Pereira *et al.* 2002).

Phylogeography is probably entering a new era. The more traditional approach of using molecular data to investigate a species' phylogeography is now complemented by recent estimates of palaeo-distribution obtained by modelling. By adding a valuable spatial-geographic component, phylogeographic inferences are clearly improved (Moussalli *et al.* 2009).

4.3.3 Implications for conservation

This chapter results have several direct consequences for the conservation of the rare *B. barbastellus*. First, I propose the existence of four major conservation units, Morocco, Iberia, Italy through to England, and the Balkans through to central and eastern Europe. Second, because high haplotype diversity was found in all of the sampled regions, all populations carry unique gene pools with need of protection. This pattern suggests that genetic drift, rather than gene flow, is the most relevant factor in shaping the spatial distribution of genetic variability (Knowles *et al.* 2007). An exceptional high number of unique haplotypes were found in Iberia, and so these populations should be protected with particular care. Also of note, high genetic diversity was found on the Isle of Wight (off southern England), although the majority of the analysed English samples came from that area, thus limiting interpretations about the extent of genetic diversity in England. Nevertheless, its populations are linked to the major colonisation event that resulted in bats colonising mainland England. To lose those populations could compromise an important source of genetic diversity in that region. Finally, despite *B. barbastellus* being dependent upon mature deciduous forests, their foraging behaviour varies considerably over different landscapes and hence countries. For example, the average home range of individual *B. barbastellus* in one area of Germany is reported to be 403 ha (Hillen *et al.* 2009), while in one region of southern England it reaches 982 ha (Davidson-Watts, pers. comm.). This means that although deciduous forests are the primary habitat to preserve, the area needed for the effective conservation of *B. barbastellus* would require prior knowledge of the local population needs and foraging behaviour over the

different seasons of the year, thus stressing the development of local studies for an effective conservation policy.

4.5 Supplementary material

Haplotype	N	Countries	10	21	30	42	45	48	52	55	105	144	147	174	179	204	234	243	297	312	315	321	345	384	390	408	411	428	487
1	3	Pt	A	C	G	T	T	C	T	G	C	G	T	T	T	C	T	T	G	C	A	C	G	C	C	A	G	C	T
2	2	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
3	5	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
4	2	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
5	1	Pt	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
6	1	Pt	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G
7	1	Pt	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
8	1	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
9	1	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
10	1	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
11	4	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12	2	Pt	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	1	Pt	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14	1	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	1	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
16	1	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
17	9	It	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	T	.	.	.	.
18	1	It	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	T	.	.	.	.
19	18	It	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	T	.	.	.	.
20	1	It	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	T	.	.	.	.
21	1	It	.	.	A	.	.	T	.	A	.	.	.	C	.	T	.	C	.	.	.	.	A	T	.	.	.	.	.
22	11	En,Gr,Bl, Sl,Hr	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
23	1	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	.	.	.	.
24	1	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	.	.	.	.
25	2	Gm	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
26	1	Bl	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
27	1	Bl	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
28	1	Hr	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
29	4	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	G	.	.	.
30	2	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	.	.	.	.
31	1	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	G	.	.	.
32	1	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	G	.	.	.
33	1	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	G	.	.	.
34	2	En	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	G	.	.	.
35	1	En	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
36	1	Sl	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
37	1	Sl	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	.	.	.	.
38	1	Sl	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.

Haplotype	N	Countries	10	21	30	42	45	48	52	55	105	144	147	174	179	204	234	243	297	312	315	321	345	384	390	408	411	428	487
39	1	Sl	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	A	.	G	.	A	.	.	.	A	.	.
40	1	Sl	.	.	.	.	.	.	.	A	.	.	.	.	C	T	C	C	.	.	G	.	A	.	.	.	A	.	.
41	1	Sl	.	T	.	C	.	.	.	A	.	.	.	.	.	T	.	C	.	.	G	.	A	.	.	.	A	.	.
42	1	Mr	.	.	.	.	C	.	.	A	.	A	.	.	.	T	.	C	.	.	.	T	A	.	.	.	.	.	.
43	1	Mr	.	.	.	.	.	.	.	A	.	A	.	.	.	T	.	C	.	.	.	T	A	.	.	.	.	.	.
44	2	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
45	1	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
46	1	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
47	1	Sp	.	.	.	.	.	.	.	A	.	.	C	.	.	T	.	C	.	T	.	.	A	.	.	.	.	.	.
48	1	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.
49	1	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.

Table S4.1 – Polymorphisms for the concatenated sequences. From 1 to 1140bp corresponds to the *cyt b* and from 1141 to 1437bp to the D-loop fragment. Number of samples included in each haplotype is also indicated (N). Pt, Portugal; It, Italy; En, England; Gr, Greece; Bl, Bulgaria; Sl, Slovenia; Hr, Hungary; Gm, Germany; Sp, Spain.

Haplotype	Countries	489	490	495	498	515	538	612	624	633	642	663	681	687	690	693	694	713	801	819	823	858	897	900	912	933	945
1	Pt	G	A	G	A	A	G	G	G	T	T	C	A	A	T	G	T	C	T	C	T	C	G	T	T	G	G
2	Pt	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
4	Pt	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	Pt	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
6	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
7	Pt	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	Pt	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	Pt	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
11	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
12	Pt	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
14	Pt	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	Pt	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16	Pt	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
17	It	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.	.	.	.	C	.	A	C	.	.	.
18	It	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.	.	.	.	C	.	A	C	.	.	.
19	It	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.	.	.	.	C	.	A	C	.	.	.

Haplotype	Countries	489	490	495	498	515	538	612	624	633	642	663	681	687	690	693	694	713	801	819	823	858	897	900	912	933	945
20	It	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.	.	.	.	C	.	A	C	.	.	.
21	It	A	.	.	.	.	.	.	.	.	C	T	G	.	.	.	.	.	.	.	.	.	A	C	.	.	.
22	En,Gr,Bl, Sl,Hr	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
23	En	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
24	En	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
25	Gm	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
26	Bl	.	G	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
27	Bl	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
28	Hr	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	C	A	.
29	En	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
30	En	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	C	.	A	C	.	.	.
31	En	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
32	En	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	C	.	A	C	.	.	.
33	En	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
34	En	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	C	.	A	C	.	.	.
35	En	.	.	.	.	G	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
36	Sl	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
37	Sl	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
38	Sl	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
39	Sl	.	.	.	G	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
40	Sl	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
41	Sl	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	T	.	T	T	C	.	A	.
42	Mr	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.
43	Mr	.	.	.	.	.	.	.	.	.	C	.	.	T	.	.	.	G	.	.	.	.	A	C	.	.	.
44	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
45	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
46	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
47	Sp	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	A	C	.	.	.
48	Sp	.	.	.	G	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
49	Sp	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table S4.1 – (cont.). Polymorphisms for the concatenated sequences. From 1 to 1140bp corresponds to the *cyt b* and from 1141 to 1437bp to the D-loop fragment. Number of samples included in each haplotype is also indicated (N). Pt, Portugal; It, Italy; En, England; Gr, Greece; Bl, Bulgaria; Sl, Slovenia; Hr, Hungary; Gm, Germany; Sp, Spain.

Haplotype	Countries	984	1012	1020	1023	1047	1089	1119	1126	1140	1141	1142	1144	1169	1197	1236	1240	1281	1305	1321	1431	1439
1	Pt	A	A	C	G	C	T	G	C	A	G	T	C	A	C	A	G	G	G	C	T	C
2	Pt	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
3	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
5	Pt	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
7	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
8	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	Pt	.	.	.	.	.	.	.	.	.	-	G	T	.	.	.	.	.	.	.	C	.
10	Pt	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
11	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
12	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
14	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16	Pt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
17	It	.	.	.	A	.	.	.	.	G	.	.	.	.	A	.	.	.	.	T	.	.
18	It	.	.	.	A	.	.	.	.	G	.	.	.	.	A	.	.	.	.	T	C	.
19	It	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.
20	It	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	T	C	.
21	It	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	.
22	En,Gr,Bl, Sl,Hr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
23	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
24	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
25	Gm	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
26	Bl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
27	Bl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
28	Hr	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
29	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
30	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.
31	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
32	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
33	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-
34	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
35	En	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
36	Sl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
37	Sl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
38	Sl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
39	Sl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
40	Sl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
41	Sl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
42	Mr	.	.	.	.	T	.	.	T	.	.	.	.	.	.	G	-	.	.	.	.	.

Haplotype	Countnes	984	1012	1020	1023	1047	1089	1119	1126	1140	1141	1142	1144	1169	1197	1236	1240	1281	1305	1321	1431	1439
43	Mr	.	.	.	.	.	.	.	T	.	.	.	.	.	.	G	-	.	.	.	.	.
44	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
45	Sp	.	.	.	.	.	.	.	.	.	-	G	T	.	.	.	.	.	.	.	C	.
46	Sp	.	.	.	.	.	.	.	.	.	-	G	T	.	.	.	.	A	.	.	C	.
47	Sp	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.
48	Sp	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
49	Sp	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table S4.1 – (cont.) Polymorphisms for the concatenated sequences. From 1 to 1140bp corresponds to the *cyt b* and from 1141 to 1437bp to the D-loop fragment. Number of samples included in each haplotype is also indicated (N). Pt, Portugal; It, Italy; En, England; Gr, Greece; Bl, Bulgaria; Sl, Slovenia; Hr, Hungary; Gm, Germany; Sp, Spain.

CHAPTER 5

Predicted impact of climate change on European bats in relation to their biogeographic patterns

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Abstract

1. There has been considerable recent interest concerning the impact of climate change on a wide range of taxa. However, little is known about how the biogeographic affinities of taxa may affect their responses to these impacts. My main aim was to study how predicted climate change will affect the distribution of 28 European bat species grouped by their biogeographic patterns as determined by a spatial Principal Component Analysis.

2. Using presence-only modelling techniques and climatic data (minimum temperature, average temperature, precipitation, humidity and daily temperature range) for four different climate change scenarios (IPCC scenarios ranging from the most extreme A1FI, A2, B2 to the least severe, B1), The potential geographic distribution of bat species in Europe grouped according to their biogeographic patterns is predicted for the years 2020-2030, 2050-2060 and 2090-2100.

3. Biogeographic patterns exert a great influence on a species' response to climate change. Bat species more associated with colder climates, hence northern latitudes, could be more severely affected with some extinctions predicted by the end of the century. The Mediterranean and Temperate groups seem to be more tolerant of temperature increases, however their projections varied considerably under different climate change scenarios.

4. Scenario A1FI was clearly the most detrimental for European bat diversity, with extinctions and declines in occupied area predicted for several species. The B scenarios were less damaging and even predicted that some species could increase their geographical ranges. However, all models only took into

account climatic envelopes whereas available habitat and species interactions will also probably play an important role in delimiting future distribution patterns.

5. *Synthesis and applications:* The models may only generate 'best case' predictions about future changes in the distribution of European bats. Nevertheless, prior knowledge of areas that will potentially be more affected by climate change, as well as the identification of areas with potential to harbour higher levels of biodiversity, would greatly help the establishment of suitable management policies. The role of protected areas could be even more enhanced as sources of offspring and individuals for dispersal and colonisation of new areas.

5.1 Introduction

During this first decade of the 21st century, climate change has become a frequently discussed issue, with coverage ranging from the more general media to more specialized scientific publications. A number of species have already been affected by recent climate change, with effects on phenology, geographical range or even local survival documented (Pounds *et* al. 1999; Parmesan & Yohe 2003; Root *et al.* 2003; Sanz *et al.* 2003; McMahon & Burton 2005). Recent studies have focused on the potential impact of climate change on global biodiversity (Araújo & Rahbek 2006), raising great concerns over the future of a range of animal (Araújo *et al.* 2006; Huntley *et al.* 2008) and plant species (McLachlan *et al.* 2005; Kirilenko & Sedjo 2007).

One of the major impacts of climate change may be the movement of populations from their original locations to new and unoccupied areas. In this process, local extinctions may occur and populations may become highly fragmented (Thomas *et al.* 2004). If these situations persist over long periods of time, it is likely that other conservation issues may arise. The extinction of species in their glacial refugia and their survival in unconnected populations may cause depletion of genetic variability and high levels of inbreeding (Ezard & Travis 2006), thus also compromising the survival of those remaining populations.

When studying species' responses to future climate change, it is important to model several future scenarios, in order to cover how each species responds to a range of climate change projections (Araújo *et al.* 2006; Beaumont *et al.* 2007). The Intergovernmental Panel on Climate Change (IPCC) has developed a set of climate change scenarios that include a wide variety of possible socio-

economic projections (Nakicenovic & Swart 2000). 'Worst case' scenarios generate more severe projections, with annual average temperatures rising up to 5.8°C by the end of the century in some regions, while other scenarios are less severe. None predicts a decrease in average temperature in comparison with present conditions.

Bioclimatic modelling is the primary tool used when simulating climate change projections (Beaumont *et al.* 2007). Climate envelopes represent the ecological conditions a species requires for its survival without taking into account any biotic interactions, such as competition with other species or other individuals, predation, and changes in food availability (Thomas *et al.* 2004). Several methods have already been developed and assayed to predict distributions under different climates, such as using Pleistocene data to build models for current distribution (Martínez-Meyer *et al.* 2004; Hijmans & Graham 2006). However, it is usual for future climate change studies to cluster their results by taxa (Araújo *et al.* 2006) or species are simply grouped into a single dataset (Thomas *et al.* 2004). This may result in great variability in the output models, because species with different biogeographical origins may have different responses to climate change. Consequently, when building species richness models it may not detect relevant conservation problems for biogeographic groups that are less representative of the taxa of interest. Furthermore, because mammals are very diverse in terms of body size, morphology and ecology they are not expected to react to climate change in a uniform manner (Scheel *et al.* 1996).

In this study, I focused on the response of 28 European bat species to climate change. Bats constitute one the most diverse mammal groups in Europe yet

many species are also threatened (Mitchell-Jones *et al.* 1999). Their diversity comprises several biogeographic groups (Horáček *et al.* 2000) with a widespread distribution in Europe, covering all the major biomes from the warmer Mediterranean to the colder Boreal and Alpine regions. Despite their diversity and wide distribution, bat responses to climate change have been little studied although Burns *et al.* (2003) predicted that bats could be among the taxa most affected by climate change in the U.S.A.

The main aim of this study was to categorise bats into biogeographic groups that currently occupy similar climatic conditions and to predict the response of each biogeographic group to a range of projected climate change scenarios until the end of the 21st century. To achieve these aims, I investigated the impact of climate change on the spatial patterns of species richness for each biogeographic group using bioclimatic envelopes to build predictive models and determined the range shift from their current distribution to their future potential occupied areas.

5.2 Methods

5.2.1 Study area

The study area included all of mainland Europe (west of Caucasus), U.K. and Ireland, all major Mediterranean islands and part of north Africa, covering land between the coordinates 71°31'N; 33°30'N; 10°45'W; 45°33'E. The study area therefore included the geographical range of all European near-endemic bat species (Mitchell-Jones *et al.* 1999), as well as the range of the most common European species. Europe's climate can be characterized overall as temperate although considerable variations exist, with a Mediterranean climate

dominating in the south (with hot summers and mild winters) whereas in the north and in mountainous regions the climate is considerably colder and more humid (Huntley *et al.* 2007).

5.2.2 Species data, climatic variables, future scenarios and spatial conversion

All presence data were obtained from Mitchell-Jones *et al.* (1999) available at the European Environment Agency website (http://eunis.eea.europa.eu). Several cryptic species have been discovered more recently (Barratt *et al.* 1997; Mayer & von Helversen 2001; Ibañez *et al.* 2006; Mayer *et al.* 2007), although data from these species were not included here because their distributions are still poorly understood. In total 28 bat species were considered for study, assuming a uniform distributional confidence for all of them. Species presence data varied between 71 known locations for *Nyctalus lasiopterus* and 1098 for *Myotis daubentonii* (average 522±322 locations), covering the majority of each species' known distribution in Europe.

Five climatic variables (average temperature (°C), minimum temperature (°C), daily temperature range (°C), relative humidity (%) and monthly precipitation (mm/month)) were chosen to model future bat distribution for four time periods: 1961-1991 (representing present climate), 2020-2030, 2050-2060 and 2090-2100. These variables were downloaded for four IPCC future scenarios as follows. A1FI (where FI stands for fossil fuel intensive) represents a globalized world with intensive economic growth sustained by the intensive use of fossil fuel; the scenario A2 is also driven by economic growth although at regional scale, creating a diversified political and social world; the B1 scenario is clearly

the "greenest" of all projections, with high levels of environmental and social consciousness and a global sustained development; B2 is the "mixed green bag" scenario, with only a regional steady growth and social awareness (Fig. 5.1; Nakicenovic & Swart 2000).

For spatial conversion of climatic data, two datasets with monthly data in a compressed format were downloaded from the Tyndall centre (Mitchell *et al.* 2004): the historical climate dataset (CRU TS 2.1 - http://www.cru.uea.ac.uk/~timm/grid/CRU_TS_2_1.html) spanning from 1901 to 2002 and the future climate dataset (TYN SC 2.0 - http://www.cru.uea.ac.uk/~timm/grid/TYN_SC_2_0.html) with climate prediction grids from 2001 to 2100, both with a resolution of 0.5° (~55km) meaning that there was a total of 5965 cells for the study area. The final map for each variable and scenario is the result of averaging the monthly grids of each analyzed period into a single map.

All operations were automated with a script made in Python programming language and incorporated as a toolbox in ArcGIS 9.2 (ESRI, Redlands, CA, USA).

5.2.3 Determination of bat biogeographic groups

In order to determine bioclimatic regions occupied by assemblages of bat species a spatial Principal Components Analysis (sPCA) was calculated using current climatic variables, where each pixel in the map was the dependent variable and climatic values constituted the independent variables (Sillero *et al.* 2009). First, the variables to include in the sPCA were chosen through analysis of their correlation matrix to avoid multicolinearities. Afterwards, each

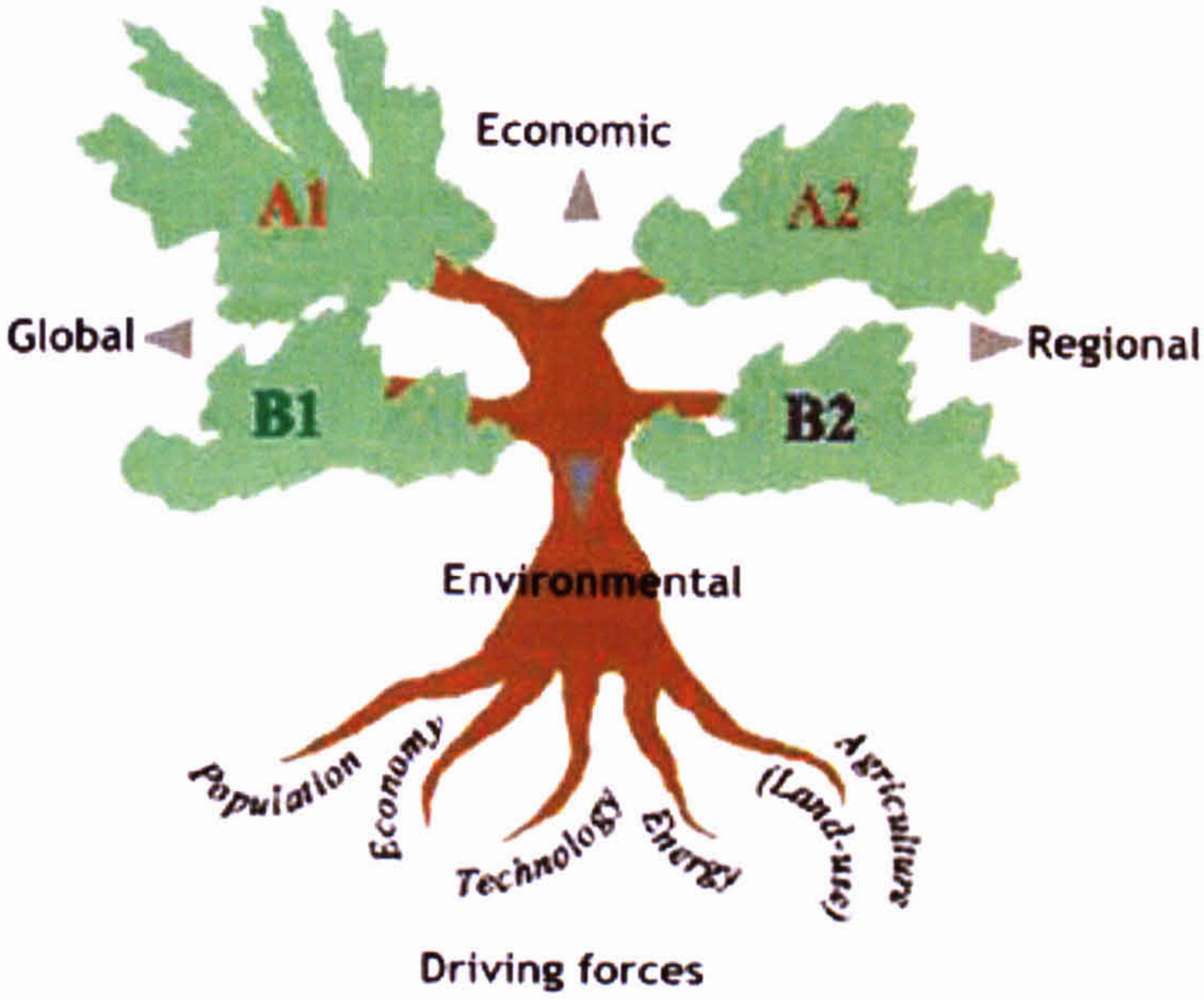

Figure 5.1 – Representation of the main climate change scenario families adapted from Nakicenovic & Swart (2000).

pixel was scored from the obtained sPCA components (or axis) and was represented in a composite map where each chosen component had a RGB colour. Consequently the colour of each pixel was a representation of the vectorial location within the sPCA. Additionally, species' locations were intersected with the previous composite map and obtained values of the sPCA axes were then used to calculate a PCA for the 28 bat species in study and subsequently determine biogeographic group for each species. All subsequent modelling and projections were done using this biogeographic grouping of species. All spatial and geographical statistics were done in ArcGIS 9.2 using

the PCA extension in the toolbox. The PCA for the 28 bat species was calculated in SPSS v15.0 (SPSS Inc., Chicago, Il).

5.2.4 Modelling procedure and testing

I chose to model using a presence-only technique because there is no reliable absence data and the elusive and nocturnal behaviour of bats adds even more uncertainty to absences. I used a maximum entropy modelling technique (Maxent 3.2.1; http://www.cs.princeton.edu/~schapire/maxent) that seems to have very good performance when compared with other methods (Elith *et al.* 2006) for past, present and future conditions, even when sample size is low (Elith *et al.* 2006; Hijmans & Graham 2006; Martínez-Freiría *et al.* 2008). Maxent is a machine-learning process that uses a statistical mechanics approach and estimates the range of a species by finding the maximum entropy distribution (i.e. closest to the uniform) given the constraint that the expected value for each variable closely matches the empirical average of the set of occurrence data.

Models were run in autofeatures with a maximum of 1000 iterations and were tested with Receiver Operated Characteristics (ROC) plots to evaluate their predictive ability. The area under curve (AUC) of the ROC analysis provides a single measure of model performance (Liu *et al.* 2005) and ranges from 0.5 (randomness) to 1 (perfect discrimination), where a score higher than 0.7 is considered a good model performance (Fielding & Bell 1997). Seventy-five percent of the presence data were randomly chosen to train the models while the remaining 25% were used to test them. The average value of AUC for all species within each biogeographic group was calculated as well as their

maximum and minimum values. In addition, the percentage of each variable's contribution to the model was determined.

5.2.5 Combining different species models and thresholds definition

The output maps from Maxent classify each pixel with a probability of occurrence between 0 and 1. The threshold value above which the species is considered present was selected in the ROC plot as the point where the sum of sensitivity and specificity is maximised (Liu *et al.* 2005). Afterwards, according to these thresholds all maps were reclassified to display areas of probable presence and absence for the species. Subsequently, the binary maps from each species' biogeographic group were added, thus producing species richness maps for each modelled scenario and time period. All analysis was carried out in ArcGIS 9.2.

5.2.6 Analysis of each biogeographic group's occupied area

In order to determine if predicted suitable areas in the future would increase or decrease in relation to current potential areas, the ratio between each future projection and the present potential occupied area was calculated and converted into a percentage of variation in relation to the latter value. Similarly, it was intended to determine if in the future projections bats would still occupy their present distribution. For this the proportion of projected suitable area that overlapped with the current distribution was calculated. Results were then averaged for each biogeographic group and scenario with maximum and minimum values also determined. Mann-Whitney U tests were also used to

check if the predicted areas occupied in the future that overlapped with the present distribution varied significantly among the biogeographic groups.

5.3 Results

5.3.1 Determination of biogeographic groups

The sPCA was calculated using three climatic variables: average temperature, daily temperature range and precipitation (Table 5.1). The other two climatic variables were not included in this analysis because they were highly correlated with the other variables used (correlation matrix values higher than 0.9 between relative humidity and precipitation and between minimum temperature and daily temperature range) and explained less of the variation than the input variables with which they were correlated.

A map that combined two sPCA components was produced (with 93.67% of cumulated variance explained, first axis score=1.8 and second axis score=1.01; Fig. 5.2). A PCA was then plotted for the 28 bat species under study by

	PCA1	*PCA2*
% variation	*60*	*33.67*
Eigenvalues	*1.8*	*1.01*
Average temperature (°C)	*0.846*	*-0.093*
Daily temperature range (°C)	*0.811*	*-0.177*
Precipitation (mm)	*-0.157*	*0.986*

Table 5.1 – Principal Component matrix for the selected climatic variables.

Figure 5.2 – Composite map combining two sPCA axis using three climatic variables (see Results). Areas represented in red were associated with warmer climates while blue areas represented colder climates. The map identified three general bioclimatic regions in Europe: the Boreal, in blue and dark colours, including Scandinavia, the Alps/Carpathians, the Pyrenees and part of Scotland; the Temperate Humid Zone located in central and eastern Europe, most of the U.K and small areas in northern Iberian Peninsula and in Turkey; and the Mediterranean area, mainly represent by red colours, and located in southern Europe.

intercepting each species' distribution with the sPCA axes (Fig. 5.3). In this plot it is possible to distinguish the three biogeographic groups that can be linked to the bioclimatic regions identified in the sPCA map (Fig. 5.2). Four species can be grouped in the Boreal biogeographic zone, 10 in the Temperate Humid Zone (hereafter called Temperate) and 14 in the Mediterranean (Fig. 5.3).

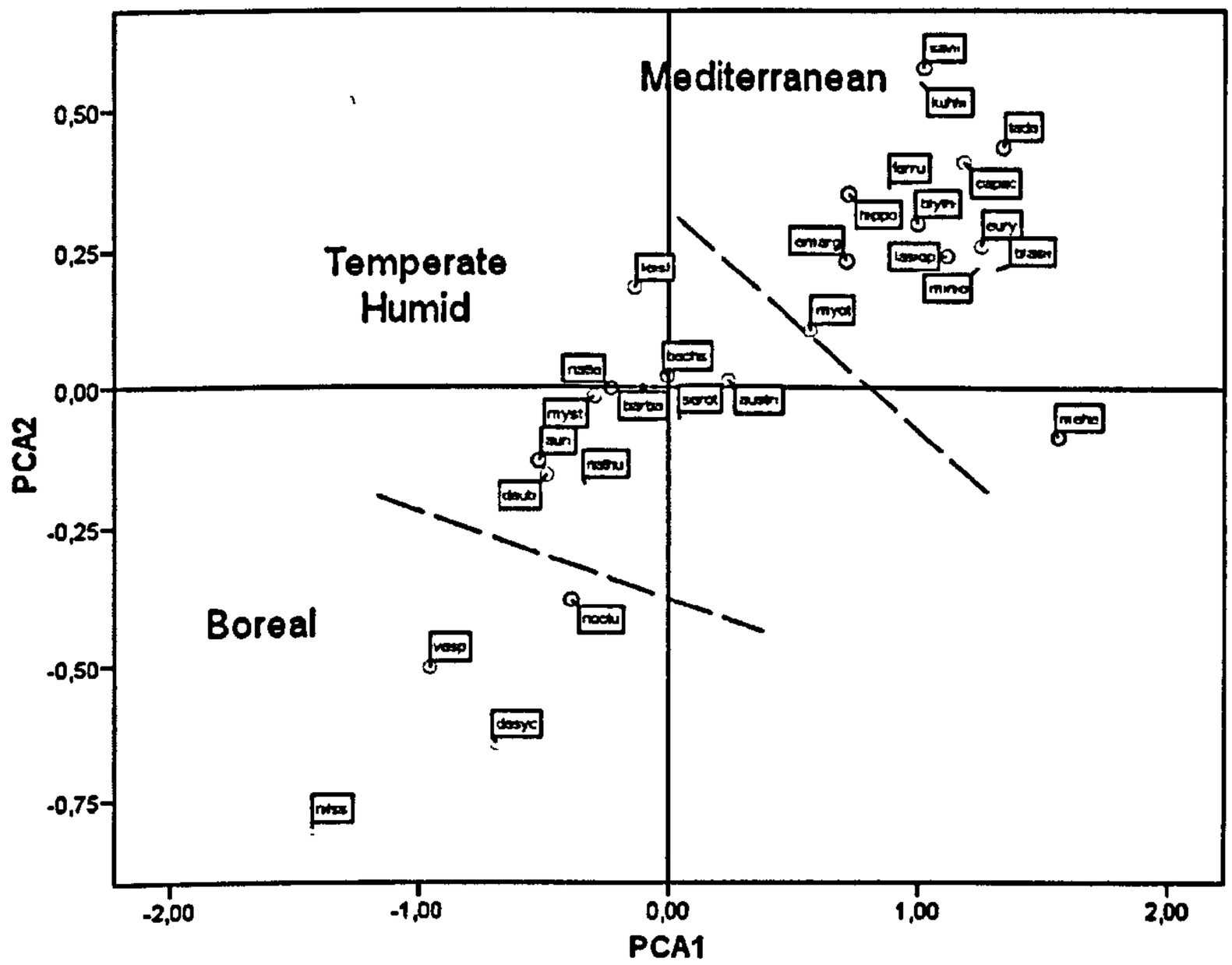

Figure 5.3 – PCA plot of the 28 bat species in study using the same three climatic variables as in the sPCA (see Suplementary material S.1). The dashed lines separate each biogeographic group. *Rhinolophus blasii*: blasii; *Rhinolophus euryale*: eury; *Rhinolophus ferrumequinum*: ferru; *Rhinolophus hipposideros*: hippo; *Rhinolophus mehelyi*: mehe; *Myotis bechsteinii*: bechs; *Myotis blythii*: blythi; *Myotis capaccinii*: capac; *Myotis dasycneme*: dasyc; *Myotis daubentonii*: daub; *Myotis emarginatus*: emarg; *Myotis myotis*: myot; *Myotis mystacinus*: myst; *Myotis nattereri*: natte; *Pipistrellus kuhlii*: kuhlii; *Pipistrellus nathusii*: nathu; *Hypsugo savii*: savii; *Nyctalus lasiopterus*: lasiop; *Nyctalus leisleri*: leisl; *Nyctalus noctula*: noctu; *Eptesicus nilssonii*: nilss; *Eptesicus serotinus*: serot; *Vespertilio murinus*: vesp; *Barbastella barbastellus*: barba; *Plecotus auritus*: auri; *Plecotus austriacus*: austri; *Miniopterus schreibersii*: minio; *Tadarida teniotis*: tada.

5.3.2 Predictive modelling, validation and testing

ROC plots exhibited very similar AUC values between training and test data although values were slightly lower for the latter (Table 5.2). Moreover, all AUC values (including registered minimums) show that the models had a very good predictive power with AUC values always higher than 0.79.

	Boreal	Temperate	Mediterranean
Daily temperature range (°C)	7.75 (5.59-9.7)	7.59 (5.48-10.11)	5.8 (3.76-8.42)
Average temperature (°C)	**33.98** (22.13-47.21)	**18.39** (11.07-27.29)	**24.59** (19.1-30.16)
Minimum temperature (°C)	**19.61** (8.07-31.63)	**24.84** (16.35-33.01)	4.82 (2.62-7.22)
Monthly precipitation (mm)	13.2 (11.54-15.09)	**21.9** (19.04-24.67)	**18.99** (16.44-22.02)
Relative humidity (%)	**25.47** (20.92-31.62)	**28.27** (23.07-34.82)	**45.79** (40.43-51.86)
Training AUC	0.86 (0.83-0.92)	0.84 (0.8-0.91)	0.9 (0.84-0.95)
Testing AUC	0.83 (0.77-0.91)	0.83 (0.79-0.91)	0.88 (0.8-0.94)

Table 5.2 - Average (minimum–maximum) percentage contribution of each variable for the predictive modelling in each biogeographic group and average (minimum–maximum) training and test area under the curve (AUC). Most important variables are in bold.

5.3.3 Analysis of variable importance

The most relevant variables for the three biogeographic groups were different, hence ecological factors limiting bat distribution differed for each group (Table 5.2). There is some variability within the Boreal group but, overall, average temperature and relative humidity were the most relevant variables for modelling the distribution of this group as well as minimum temperature. The Temperate group had the greatest variability of all biogeographic groups. Nevertheless, minimum temperature and relative humidity had the greatest importance whilst average temperature and monthly precipitation seem also important for some of these bats. For the Mediterranean biogeographic group, average temperature, relative humidity and monthly precipitation were the most important factors limiting distribution, while minimum temperature (which was very important for the other two groups) had no relevance for delimiting the distribution of these bats.

5.3.4 Determination of bat diversity hot-spots from present to 2100

Currently, the highest species richness of bats in Europe is mainly located in the peninsulas of southern Europe and in southern France (Fig. 5.4a). Species

richness within the Boreal group is concentrated in the north-east of Europe with species from this group being almost absent from southern Europe (Fig. 5.4b). Species richness in the Temperate group is clearly focused in central Europe and the U.K., although high levels of richness also occur in the northern areas of the peninsulas in southern Europe (Fig. 5.4c). Despite not being the most species-rich group, the Temperate group is clearly the most widespread group, occupying the greatest area in Europe. As expected, the Mediterranean group had the highest species richness in the southern European peninsulas and in north Africa (Fig. 5.4d). This group has the largest number of species altogether although it does not occupy the largest area.

The modelling projections for each biogeographic group produced very different outcomes. The distribution of species richness in the Boreal group was strongly affected by climate change. Distribution patterns moved in a north-east direction (Fig 5.5) and scenarios A1FI and A2 had the greatest impact on bats. Although scenarios B1 and B2 had less dramatic effects, bat diversity nevertheless suffered considerable losses. Under scenario B2 it was predicted that an area connecting Scandinavia to the European mainland may be an enclave for the remaining species in this group. Scenario B1 was very similar to B2 but with more suitable areas predicted in northern Europe. To sum up, it is likely bats in the Boreal group will face serious challenges to their survival by the end of the century whichever scenario is modelled.

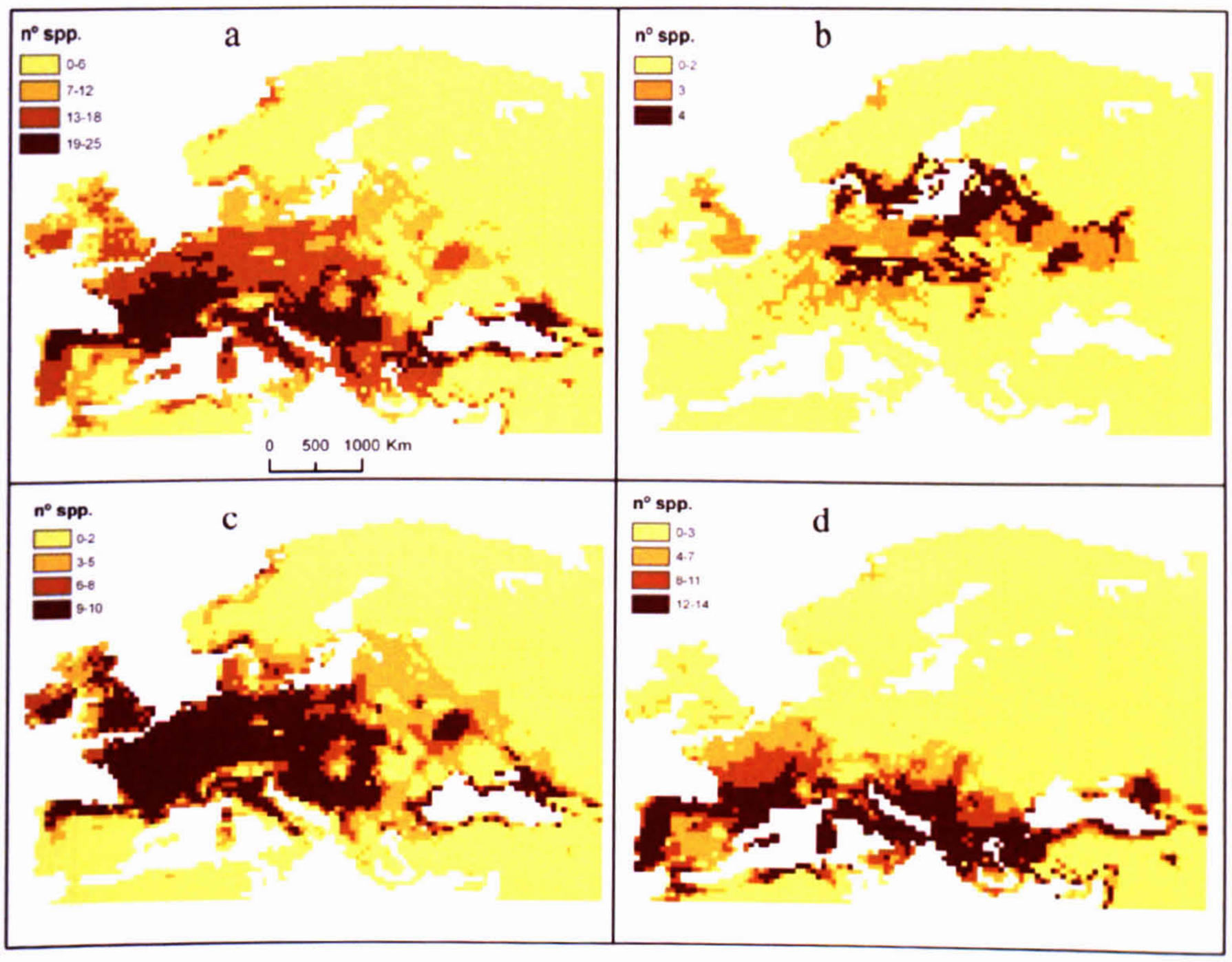

Figure 5.4 – (a) Predicted total European species richness for present distribution and for each biogeographic group: (b) Boreal, (c) Temperate and (d) Mediterranean.

The Temperate group currently occupies the largest potential area in Europe. There were no visible differences in predicted distributions amongst the different scenarios until 2050-2060 (Fig. 5.6). In fact, it is predicted a trend of species richness becoming highest initially towards the north. However, by the end of the century the different scenarios have completely different outcomes. In scenario A1FI a major reduction in the species richness of Temperate bats throughout mainland Europe is expected. Only Scandinavia and the U.K. are exceptions, with an increase from present day richness, hence they could become the most important areas for this group. Scenario A2 also predicts a

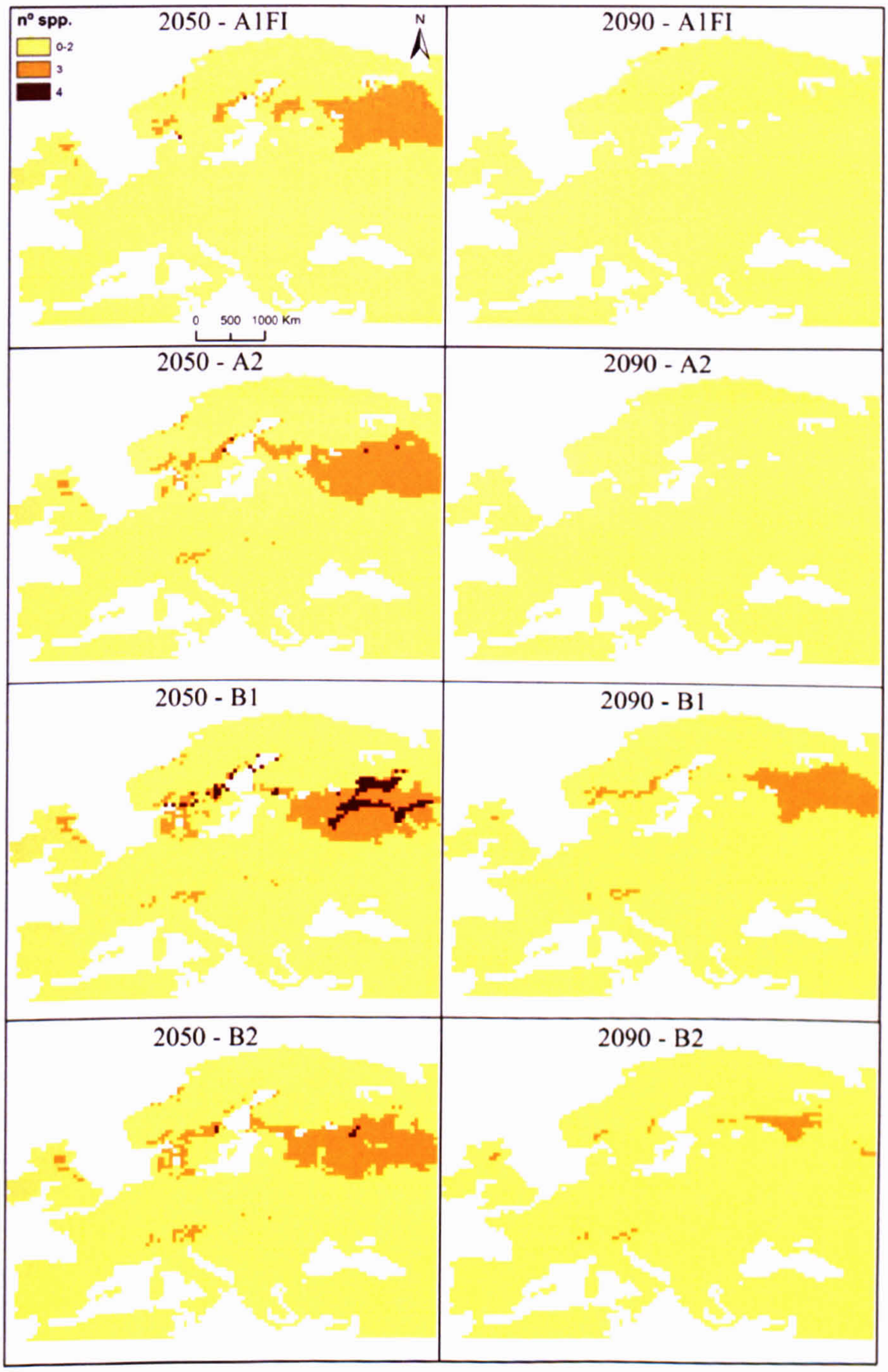

Figure 5.5 – Modelled potential distribution of bat diversity for the Boreal biogeographic group for two time periods (2050-2060 and 2090-2100) and four IPCC scenarios (A1FI, A2, B1 and B2). See Fig. 5.3 to check which bat species were included in this group.

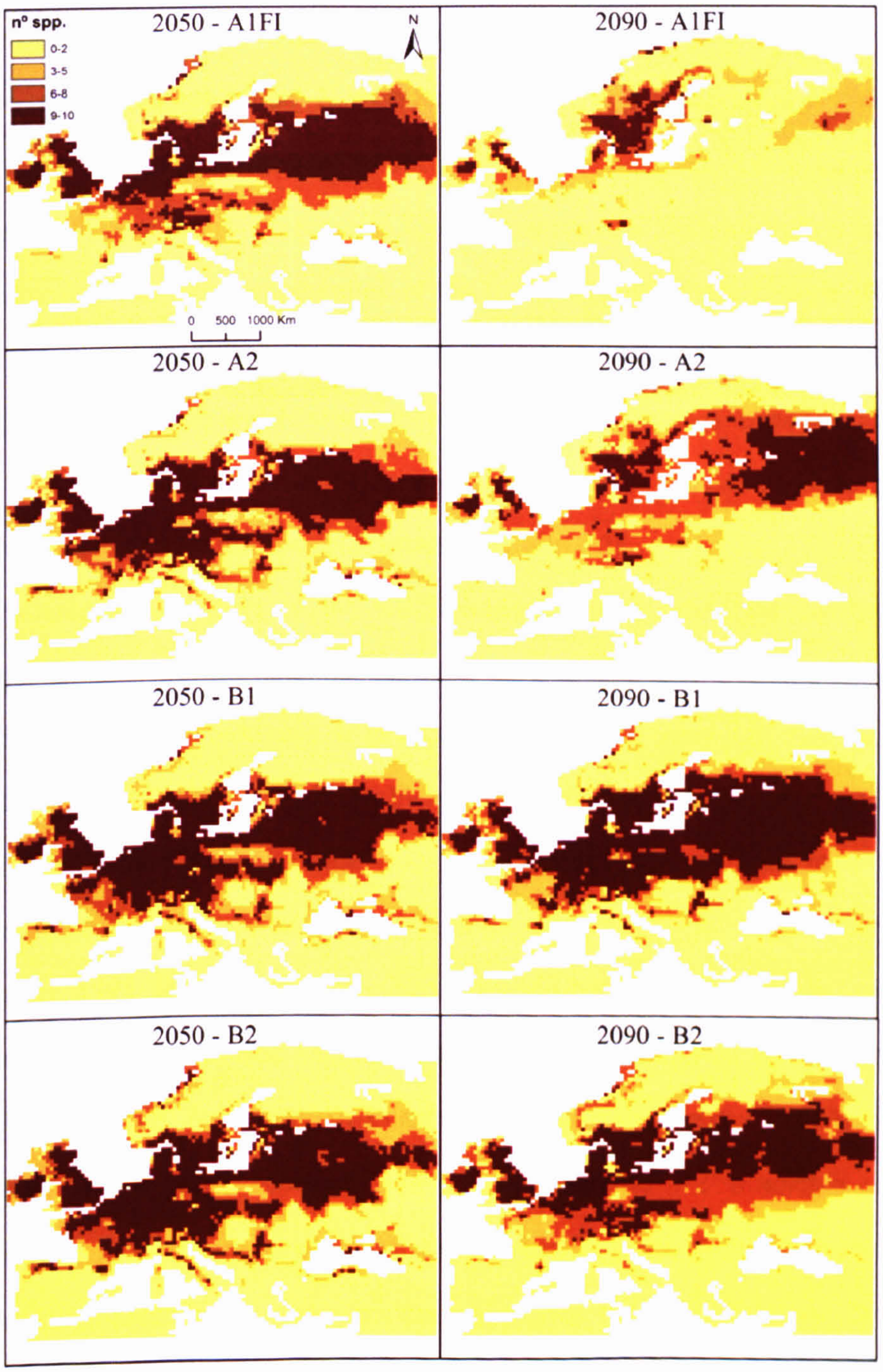

Figure 5.6 - Modelled potential distribution of bat diversity for the Temperate biogeographic group for two time periods (2050-2060 and 2090-2100) and four IPCC scenarios (A1FI, A2, B1 and B2). See Fig. 5.3 to check which bat species were included in this group.

major disappearance of European species, although it also predicted high levels of richness in northern Europe and the Alps. Again, scenarios B1 and B2 predicted the largest areas of relatively high richness with these becoming focussed in central and northern Europe. Nevertheless, both scenarios predict the almost complete disappearance of this group from southern Europe. This is more visible in the B2 scenario. Briefly, the future of these bats seems to be highly dependent on which scenario was modelled. It varies between almost total extinction (in scenario A1FI) to a widespread high level of diversity being maintained in central Europe (in scenarios B1 and B2).

It is expected that bats of the Mediterranean group could be the greater beneficiaries of climate change because they are already adapted to warm conditions. Models predicted a gradual expansion to north of their current distribution and until 2050-2060 there were no major regional extinctions predicted in their current range (Fig. 5.7). Again, however bats face more dramatic consequences of climate change by the end of the century. In scenario A1FI, major extinctions occur in southern Europe and a general movement of bat species richness moves northwards. Only some parts of Scandinavia, U.K. and northern Europe will potentially harbour high levels of diversity. Apart from scenario A1F1, only scenario A2 predicted major extinctions in the current range of Mediterranean bats, with the remaining scenarios forecasting a major expansion of this group in Europe. Overall, central and northern Europe were predicted to become highly suitable for the richness of Mediterranean bats in the future. Once more, the future of this group is dependent on which scenario is modelled. As long as the A1FI

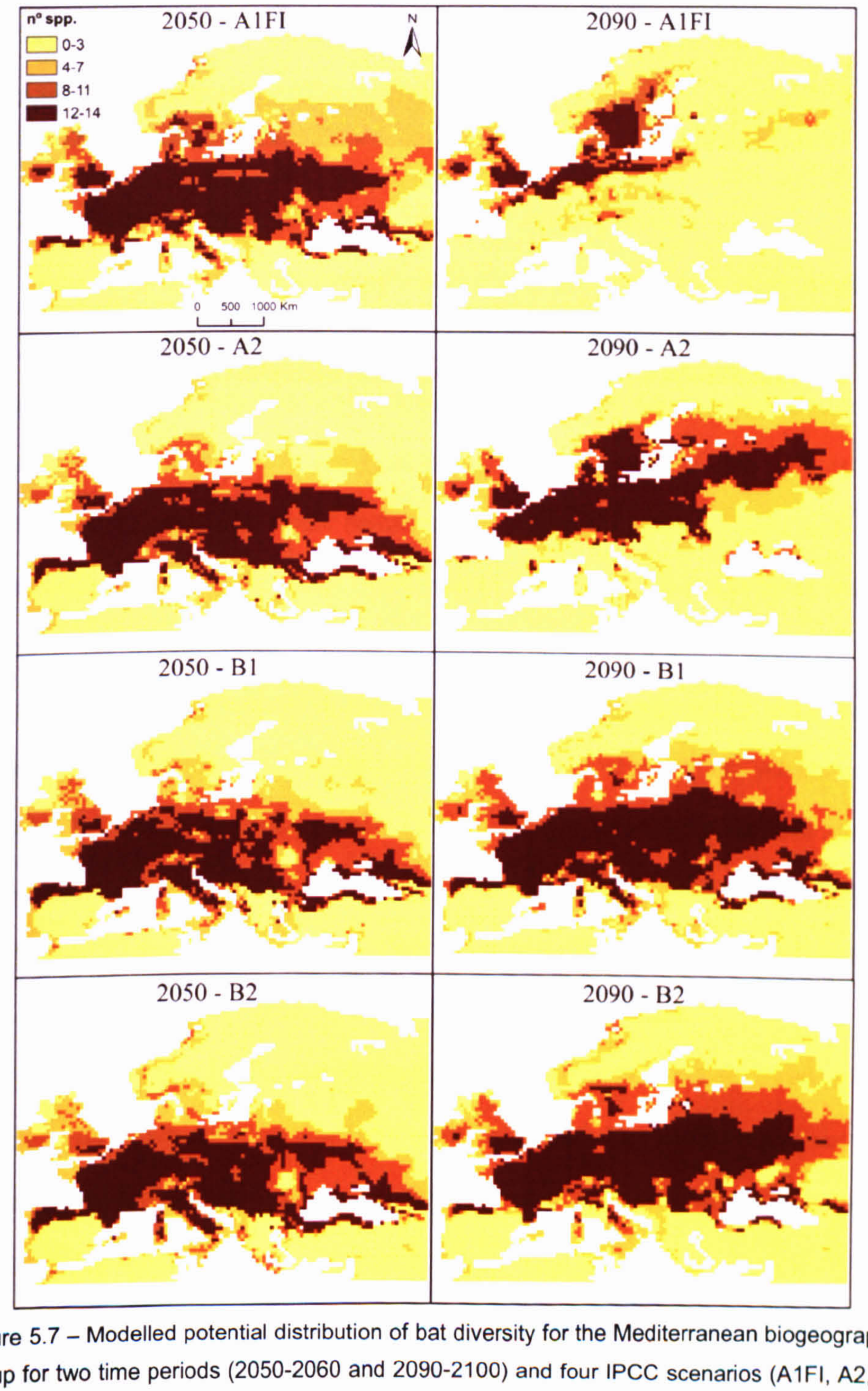

Figure 5.7 – Modelled potential distribution of bat diversity for the Mediterranean biogeographic group for two time periods (2050-2060 and 2090-2100) and four IPCC scenarios (A1FI, A2, B1 and B2). See Fig. 5.3 to check which bat species were included in this group.

scenario is avoided, it is not expected that bat diversity in this group is at serious risk if considering bioclimatic conditions only.

5.3.5 Predicted range shifts of the biogeographic groups

The three biogeographic groups generated very different predictions regarding their occupied area throughout the 21st century (Fig 5.8). Nevertheless, scenario A1FI is clearly the worst for all biogeographic groups and for the vast majority of the 28 bat species (Table 5.3). By the end of the 21st century scenario A1F1 predicted major reductions in areas occupied by bats and even some extinctions. On the other hand, scenarios B1 and B2 had least impact, although the Boreal group suffered a considerable decrease in occupied area even under these conservative models. In fact, the Boreal group will probably suffer the most severe consequences from climate change. No matter which scenario is chosen, a steady decline in the area occupied by Boreal bat species is predicted throughout the 21st century, and *Myotis dasycneme* is predicted to be at risk of extinction by 2050-2060 whichever scenario is modelled (99% reduction in occupied area by the end of the 21st century; species data available in Table S5.1, Supplementary material). The other three Boreal species (*Nyctalus noctula*, *Eptesicus nilssonii*, and *Vespertilio murinus*) also suffer major reductions in areas predicted to be suitable for occupation under all scenarios modelled.

The area predicted to be suitable for bats in the Temperate group varies according to the scenario used. Nevertheless, in general occupied areas may increase until 2050-2060 independently of the modelled scenario. By the end of

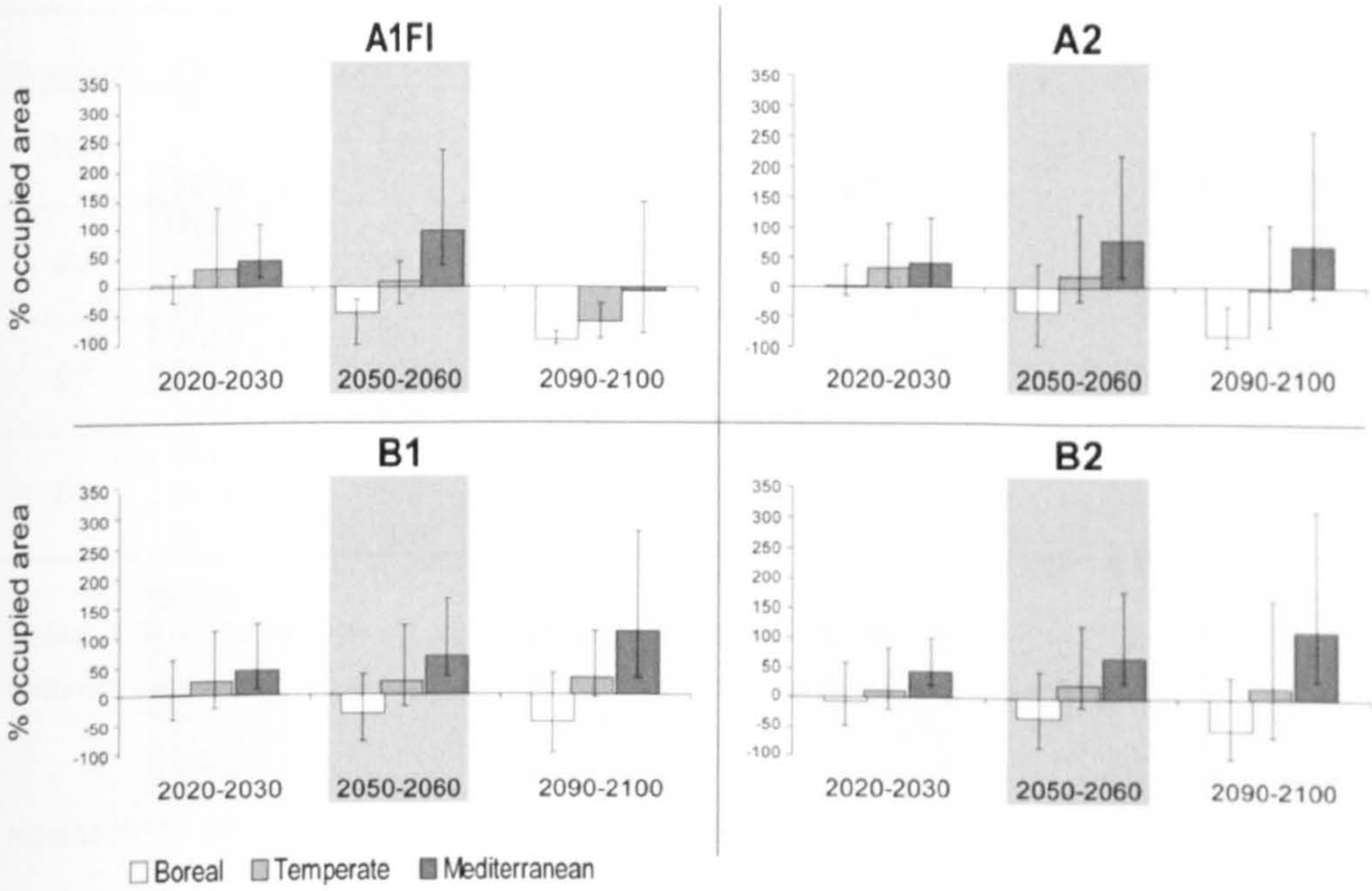

Figure 5.8 – Average variation of occupied area throughout the 21st century in relation to area currently occupied by each biogeographic group. The vertical bar indicates maximum and minimum values registered for a bat species within each group.

the century model predictions differed considerably: scenarios A1FI and A2 predicted a decrease in occupied area, whereas the B1 and B2 scenarios predicted a slight increase. *Plecotus auritus* and *Barbastella barbastellus* were the Temperate species that will probably face major reductions in their occupied area (around 90% and 62% reduction under A1FI, respectively), while *Myotis mystacinus* and *Nyctalus leisleri* had the greatest predicted increase in occupied area under all scenarios except for A1FI (up to 72% and 39% increase, respectively), where all species decrease their range.

Climate change seems to have least impact upon the area predicted to be suitable for the Mediterranean group. An increase in occupied area is predicted for the majority of species except under the A1FI scenario. Moreover, occupied area is not predicted to decrease for any species under the B1 and B2

		Boreal		Temperate		Mediterranean	
Scenario	Decade	Contracting	Expanding	Contracting	Expanding	Contracting	Expanding
A1FI	2020	25	75	10	90	0	100
	2050	100	0	40	60	0	100
	2090	100	0	100	0	71.4	28.6
A2	2020	50	50	10	90	0	100
	2050	100	0	20	80	0	100
	2090	100	0	60	40	14.2	85.8
B1	2020	25	75	10	90	0	100
	2050	100	0	20	80	0	100
	2090	100	0	20	80	0	100
B2	2020	50	50	30	70	0	100
	2050	100	0	30	70	0	100
	2090	100	0	30	70	0	100

Table 5.3 – Percentage of contracting and expanding bat species in Europe among the different biogeographic groups and modelled projections for all future scenarios.

scenarios. *Myotis blythii*, *M. myotis* and *Rhinolophus euryale* will be the most affected species with a considerable decrease in occupied area in the A1FI and A2 scenarios by the end of the century (up to a decrease of 80%, 66% and 58% respectively). *M. blythii* might even face extinction in the A1FI scenario while the majority (71.4%) of the remaining species will probably suffer a reduction in their distribution. On the other hand, *Nyctalus lasiopterus*, *Hypsugo savii* and *Tadarida teniotis* had the greatest increase in the occupied area in all scenarios (up to 194%, 229% and 222% for the B2 scenario, respectively).

How much of the current range will still be occupied in the future projections, varies considerably according to scenario (Fig. 5.9). During initial stages, the proportion of lost area in the current range is similar for all biogeographic groups for all projections (Table 5.4) with no major reductions for the majority of the species. However, for the last two modelled periods the overlapped area between current and future predicted distributions decreases the most for the Boreal group whereupon it suffers major reductions resulting in the probable disappearance from its current range whichever scenario is modelled.

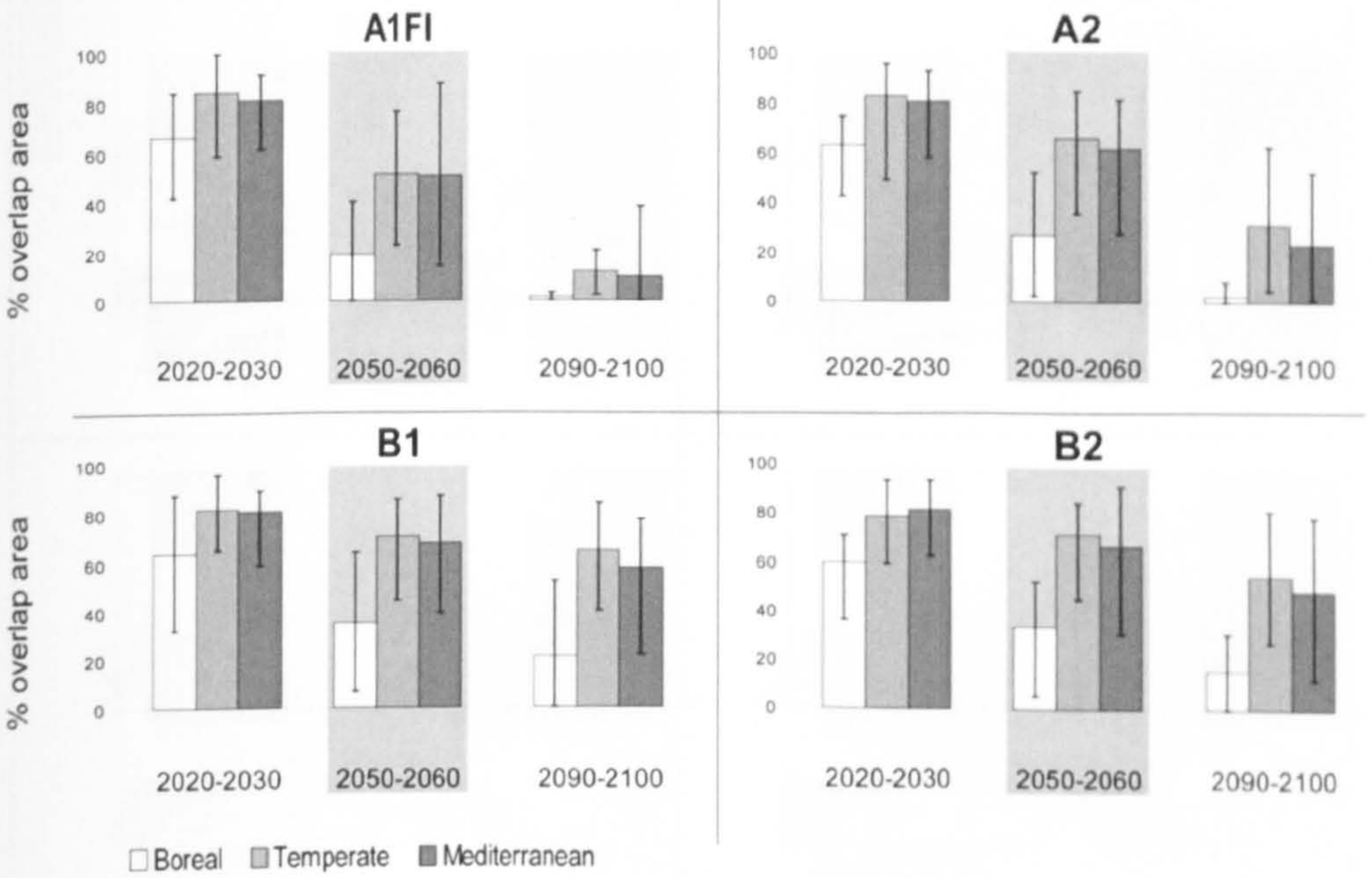

Figure 5.9 – Proportion of overlap area between projected models and the current potential distribution. The vertical bar indicates maximum and minimum values registered for a bat species within each group.

Regarding the Temperate and Mediterranean groups, there is no significant difference among them between the rate at which they contract from their current range (Table 5.4), with a more accentuated decline between 2050 and 2100. Once more the scenario A1FI is clearly the worst with major regional extinctions predicted, while the B1 and B2 scenarios predict that these groups will still occupy about half of their present range. Overall, by the end of the century there are major extinctions predicted in southern Europe with some areas loosing up to 25 species whichever scenario is modelled (Fig. 5.10). On the other hand, areas in northern Europe, British islands and Scandinavia have a potential to increase their species richness up to 24 species. Regarding

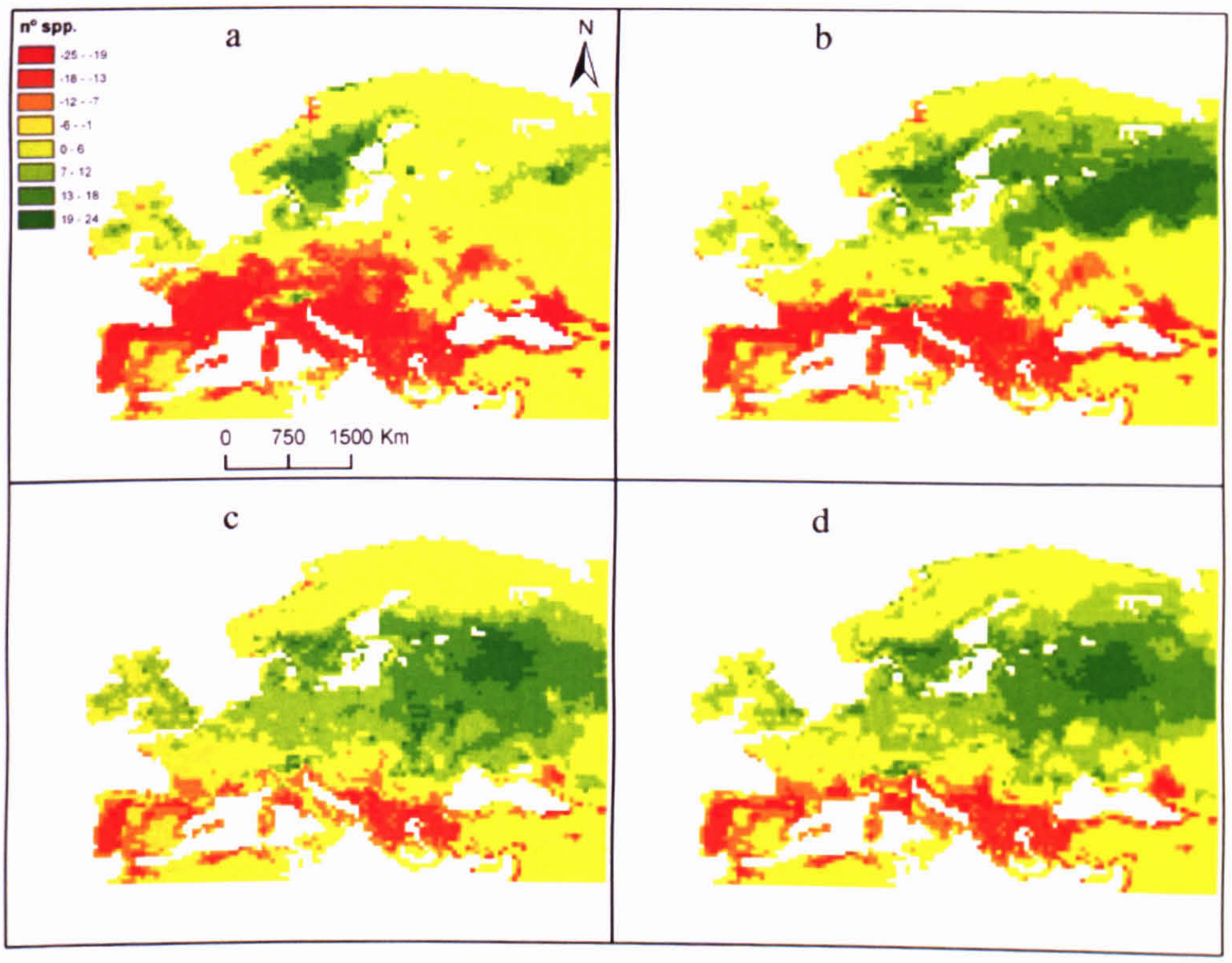

Figure 5.10 - Difference between present species richness and projections for 2090-2100 for scenarios a) A1FI, b) A2, c) B1 and d) B2.

differences among scenarios, once again the A scenarios have more species loss than B scenarios, especially in southern European peninsulas.

5.4 Discussion

5.4.1 Potential impact of climate change on European bats

This study indicated that European bats may face a serious threat with predicted climate change for the 21st century due to a rapid potential movement of their populations towards the north, a decline in their occupied area and a reduction in or disappearance from their current range. Moreover, the magnitude of that impact differs considerably for different biogeographic groups of bats. Of especial concern are northern latitude species where climate change could eliminate suitable climatic conditions whichever future scenario is

Period			Boreal	Mediterranean
2020-2030	A1FI			
		Boreal		n.s.
		Temperate	*	n.s.
	A2			
		Boreal		*
		Temperate	*	n.s.
	B1			
		Boreal		n.s.
		Temperate	n.s.	n.s.
	B2			
		Boreal		*
		Temperate	n.s.	n.s.
2050-2060	A1FI			
		Boreal		*
		Temperate	*	n.s.
	A2			
		Boreal		*
		Temperate	*	n.s.
	B1			
		Boreal		*
		Temperate	n.s.	n.s.
	B2			
		Boreal		*
		Temperate	*	n.s.
2090-2100	A1FI			
		Boreal		*
		Temperate	*	n.s.
	A2			
		Boreal		*
		Temperate	*	n.s.
	B1			
		Boreal		*
		Temperate	*	n.s.
	B2			
		Boreal		*
		Temperate	*	n.s.

Table 5.4 – Mann-Whitney U tests (*P<0.05; n.s. P>0.05) used to test for pairwise differences on the proportion of overlapped area amongst the three different biogeographic groups.

modelled. As for Temperate and Mediterranean bat species, their future seems to be more dependent on the modelled scenario. The A scenarios of a more economically driven world resulted in the biggest losses for bat species richness whereas, as expected, the more environmentally-friendly B scenarios predicted fewer losses. Nevertheless, whichever scenario is modelled there will be a reduction in bat species richness when compared to the current situation.

For this chapter, I used climate change scenario data developed in the IPCC's third assessment held in 2001. Subsequently, in 2007 the IPCC organized a fourth meeting although only recently has climatic data predicted under future climate change scenarios become available. The outcomes of the 2007 meeting resulted in the proposal of six climate change scenarios. Therefore, scenarios from these two meetings are not directly comparable. Nevertheless, for similar scenarios both assessments predicted similar climate change outcomes, showing that the degree of climate change strongly depends on the green-house gas emission level (Christensen *et al.* 2007; Ekwurzel 2007).

5.4.2 Importance of considering biogeographic groups

One of the most important outcomes of this study is the relevance of classifying species according to their biogeographic patterns. Several important climate change studies generated predictions with respect to taxonomic groups (e.g. Thomas *et al.* 2004; Araújo *et al.* 2006). I stress that it is important to take into consideration the biogeographic patterns of the species since these also reflect ecological characteristics and limitations of taxa (Cox & Moore 2005). By considering biogeography, the variability in the predictions is reduced and it is possible to determine more clearly which ecological factors are priorities for biodiversity conservation. If all bat species had been considered in a single model it would generate highly variable predictions and undetectable specific problems linked to groups associated with colder climates and northern latitudes that have lower diversity within the taxon. Such is the case of the Boreal group in the current study which is the most affected group although the one with the lowest species richness.

The classification of the 28 bats species in three large biogeographic groups is largely in agreement with results obtained using distribution data (Horáček *et al.* 2000). Consequently, there was little variability in the importance of climatic variables limiting distribution within the Boreal and Mediterranean groups. Energy, which is associated with temperature parameters in climate studies (Araújo *et al.* 2006), could be limiting the distribution of Boreal bats as suggested by Speakman *et al.* (2000). Whereas, water (relative humidity and monthly precipitation) was one of the most relevant ecological factors affecting distribution of the Mediterranean group (Russo & Jones 2003; Rebelo & Rainho 2009). The Temperate group had the greatest variability regarding which climatic factors were more relevant, probably reflecting the widespread distribution of this group. Moreover, the conjugation of these climatic variables, in particular temperature, has been acknowledged to exert a strong influence on European bat species richness patterns (Ulrich *et al.* 2007) hence supporting the selection of climatic variables as ecological predictors for bat distribution.

5.4.3 Challenges for species' survival due to climate change

The rate and magnitude of potential shifts in distribution due to climate change poses probably one of the most dramatic challenges to a species' survival prospects (Root *et al.* 2003). Many species have little or no overlap between their current and predicted range. Such species could face enhanced extinction risk, especially those near-endemic in Europe or those with limited climatic tolerance (Huntley *et al.* 2008). In more severe cases, some species may be unable to find suitable habitats or climatic conditions to survive, since each

species' ability to colonise new areas could be severely limited by their potential niche characteristics. Current studies suggest that effective species niches are conservative over time (Martínez-Meyer *et al.* 2004), meaning that when environmental conditions change dramatically outside of the ecological conditions to which populations are currently adapted, there is either migration towards new areas with suitable conditions or extinction is probable (Thomas *et al.* 2004; Hijmans & Graham 2006). Thomas *et al.* (2004) indicated that an alarming number of species may lose a part of their range and consequently become extinct. This is of special concern for polar or boreal species where it is predicted that there would be a general decline in their range with climate change (Parmesan & Yohe 2003). Additionally, these population movements may create isolated populations in their former range. The resolution of the models does not allow us to detect eventual isolated populations at a local scale, like mountain tops or valleys. Nevertheless at a broader scale, for the Boreal group in the B scenarios it is expected that populations may persist in the Alps and in some areas of Scandinavia and Scotland, although population connectivity among these regions would be difficult. For the Temperate and Mediterranean groups, only in the A1FI could some isolated populations potentially appear, especially in the Alps.

Matching phenology with future climatic conditions will also constitute one of the main pressures for bat populations, especially for example if temperature-driven gestation times become out of synchrony with food abundance (Sanz *et al.* 2003; Thomas *et al.* 2004). There are some indications that climate change is already affecting bats in Europe. For example, the Mediterranean species *Pipistrellus kuhlii* has expanded its range northwards in the past 15 years

presumably in response to increased temperatures (Sachanowicz *et al.* 2006), while parturition in *Myotis myotis* has occurred up to six months before the usual birth period in southern Spain (Ibáñez 1997). If changes in phenology and ecology are already being detected when the world has only warmed by an average of 0.6ºC, many more far-reaching effects will probably occur in response to levels predicted by the IPCC (Root *et al.* 2003). In addition, landscape changes may lead to yet greater species loss. In fact, habitat loss or alteration is currently recognized as one of the major causes of the extinction of species (Moreira & Russo 2007). This situation could become more severe when interacting with climate change. For example, Jetz *et al.* (2007) argued that between 400-900 of the world's bird species will have 50% of their current range transformed into a different habitat by 2050. Bats may be more flexible than other mammals because flight may facilitate relocation as a response to climate change (Scheel *et al.* 1996). However, the availability of roosts is one of the most limiting resources for bats (Rodrigues *et al.* 2003; Russo *et al.* 2004) and in combination with the needs for specific foraging habitats (Russo & Jones 2003; Rainho 2007), roost loss may pose an additional threat to bat survival. This may be of special concern for tree-dwelling bats since the rate of climate change may be too fast to allow the development of mature forests in the new climatically suitable areas in the north. It is not expected that large areas of mature broadleaf forest, the main habitat for the existence of tree roosts in Europe (Lewis 1995; Russo *et al.* 2004), could develop in northern Europe until the end of the century (McLachlan *et al.* 2005). Besides the disappearance of suitable roosts, climate change could modify the microclimatic condition within roosts, which could affect crucial phases of bat's life cycle such as breeding

and hibernation. The thermal conditions of roosts have a strong influence on bats' survival, because metabolic rate, evaporative water loss and gestation time are adversely affected when temperatures lay outside optimum conditions (Racey *et al.* 1987; Webb *et al.* 1995).

Since barbastelles are the main studied species throughout this thesis, I would like to draw your attention to the potential effect of climate change in this species. Like for the majority of bat species belonging to the temperate group, until 2020 it is not expected a great variation in occupied area in relation to nowadays. Only by 2050 it is expected a reduction on their extent in the A scenarios, a situation that will clearly deteriorate by the end of the century. Major reductions are expected in all scenarios but B1, making this species one of the potentially most affected by climate change in the temperate group. According to IUCN (Hutchison *et al.* 2001), currently the main threat to this bat populations' is the loss of occupied are due to a decrease in available habitat. According to this work, barbastelles could even face greater challenges for their survival in the predicted climate change.

5.4.4 Bioclimatic models as an important tool in climate change studies

Bioclimatic models provide the first approximations of the potential magnitude of the effects of climate change on species distributions (Pearson & Dawson 2003), having been used to successfully predict the potential distribution of species under both current and past conditions (Hijmans & Graham 2006). Moreover, Maxent also produces robust estimates of potential range shifts with climate change (Hijmans & Graham 2006). Overall, these predictions should be considered conservative, meaning that some omission errors could exist (the

species may exist in areas classified as unsuitable) but commission errors are unlikely (the species does not occur in areas predicted as suitable). This derives from the characteristics of Maxent: using pseudo-absences in the calculations implies that predictions are prone to overfit presence data, hence this technique is likely to reflect the natural distribution or the realized niche of taxa (Zaniewski *et al.* 2002).

Several authors have already verified that there is a great variability among projections from different future scenarios (Thuiller 2004; Araújo *et al.* 2006). As such, according to IPCC (2001) recommendations, a range of different scenarios should be used when assessing the impacts of climate change. In spite of the variability of the scenarios analysed, in all of them a trend for species richness to shift northwards was observed. This in agreement with other studies on other taxa that also predict a displacement of species richness towards areas that are nowadays cooler (Parmesan & Yohe 2003; Araújo *et al.* 2006; Huntley *et al.* 2008). Several studies have predicted an increase on potential suitable area for temperate or warmer climate species due to a warming in the cooler northern ranges. However, the majority of those works rarely extend their predictions beyond 2050 (e.g. Thomas *et al.* 2004; Araújo *et al.* 2006; Jetz *et al.* 2007). The results in this study have also shown an increase in occupied area for most of the Temperate and Mediterranean bats over that time period. Only by the end of the century was there a clear decline for almost all bats in the more economically-driven scenarios that, not surprisingly, result in the greatest changes to present climate. However, it should be also taken into account an eventual invasion of species from warmer climates, for example north African species. Indeed, some of those species

may already exist in the southern regions of the Iberian Peninsula (Ibañez *et al.* 2006). On the other hand, it can be argued that European bat species have suffered major and rapid climatic changes in the past. In fact, during the Eemian interglacial (130k – 115k years BP) or even in the Atlantic phase of the Holocene (7.5k – 5k years BP) temperatures were higher up to ca. 2ºC than present conditions (Kaspar *et al.* 2005). Moreover, the fossil record suggests that the distribution of several bat species did not suffer major changes despite the occurrence of warmer conditions during the Middle and Late Holocene (Postawa 2004). What clearly differs from the climate change future scenarios is the magnitude of temperature change. In this work, it was also shown that few changes are expected in bat distribution until mid 21st century, when temperature rises reach similar values of the Eemian and Atlantic phase of the Holocene. It is when predicted temperature rises to much higher values (up to ca. 6ºC) than those acknowledged for the aforementioned periods, that bat populations will suffer a pressure not comparable with what existed over the last interglacial climates.

5.4.5 Final remarks

As species change their geographic distribution, European protected areas will face new challenges. As some of these species succeed in colonising new areas, also new ecological relationships will be forged that can change interactions and fundamental ecosystem processes in unpredictable ways (Walther *et al.* 2002), thus it is highly probable that several ecosystems will become disrupted (Root *et al.* 2003). Protected areas could have their

relevance even more enhanced by providing a continuous source of offspring to eventually colonise more favourable areas (Huntley *et al.* 2008).

Therefore, the predicted contractions/expansions in the distribution will put more pressure on the survival of the European bats. As such, it is expected that the current conservation status of several species will suffer changes, especially for the Boreal and Temperate bats that will probably become of greater conservation concern. In fact, considering bioclimatic conditions alone, it is expected that only for a few Mediterranean species will populations expand irrespective of the scenario modelled. Overall, the number of threatened bat species is predicted to increase until the end of the current century, even without taking into account the consequences of changes in species interactions and ecosystems. Only with the implementation of climate change mitigation measures together with effective habitat management may we avoid the outcomes predicted by more detrimental future scenarios. By reacting proactively we may perhaps better manage the new conservation challenges for this century.

5.5 Supplementary material

Bioregion	Species	IUCN Status	Population Trend (2008)	2020				2050				2090			
				A1FI	A2	B1	B2	A1FI	A2	B1	B2	A1FI	A2	B1	B2
Boreal	vesp	LC	Stable	17.5	20.0	18.6	18.2	-33.3	-18.7	-11.7	-6.7	-78.4	-48.4	-17.7	-10.2
	noctu	LC	?	5.4	-5.2	11.6	-11.1	-22.6	-18.3	-14.2	-25.2	-93.7	-97.5	-14.6	-50.0
	dasyc	NT	Decrease	-28.3	-14.7	-39.8	-46.8	-99.1	-96.7	-79.9	-83.6	-99.5	-99.5	-99.6	-99.8
	nilss	LC	Stable	20.1	10.5	19.8	13.5	-27.3	-25.6	-15.1	-10.4	-93.6	-84.4	-49.2	-48.7
Temperate	auri	LC	Stable	10.8	-1.3	-20.9	-6.6	-26.7	-22.7	-19.1	2.1	-90.5	-41.5	4.6	-62.8
	austri	LC	?	37.9	72.0	64.2	50.1	33.9	80.0	77.4	85.6	-40.3	43.7	55.2	80.8
	barba	NT	Decrease	2.5	21.8	17.9	10.6	-29.5	-11.3	2.1	-3.9	-61.7	-64.9	-3.7	-21.8
	bechs	NT	Decrease	41.4	59.9	52.6	15.3	31.6	55.3	22.2	67.2	-29.9	-23.4	73.7	16.1
	daub	LC	Increase	0.0	0.5	2.5	-5.2	-7.2	-10.9	-0.1	-6.6	-56.7	-20.3	-3.1	-0.2
	leisl	LC	?	26.1	38.9	28.7	20.2	19.7	20.8	30.6	30.7	-42.8	24.6	38.8	20.6
	myst	LC	?	136.8	51.8	40.4	22.7	45.0	46.1	47.9	25.2	-59.2	37.8	72.0	70.6
	nathu	LC	?	20.8	31.6	17.3	-19.8	-26.7	4.3	22.5	-12.3	-54.3	-10.9	24.5	25.1
	natte	LC	Stable	32.3	29.5	29.3	22.5	40.2	14.2	40.8	42.3	-56.7	-0.5	37.6	53.5
	serot	LC	?	17.2	19.7	23.8	15.2	17.4	14.5	10.8	6.2	-52.2	10.2	15.0	22.7
Mediterra-nean	blasii	LC	Decrease	71.7	44.7	60.8	70.6	103.3	74.5	79.6	70.5	37.5	124.7	130.8	166.2
	blythi	LC	Decrease	17.2	17.5	11.5	22.1	38.1	17.8	42.4	33.4	-80.3	-16.9	31.3	36.8
	capac	Vu	Decrease	67.8	67.1	73.8	63.3	150.9	126.3	114.5	106.6	43.7	143.8	165.7	174.8
	emarg	LC	Stable	29.1	9.8	36.2	70.9	63.2	96.5	42.9	69.3	-33.0	46.2	92.9	89.5
	eury	NT	Decrease	41.1	45.3	32.9	34.6	67.8	73.5	42.1	65.6	-58.5	10.2	102.0	64.9
	ferru	LC	Decrease	25.5	19.6	30.6	25.1	46.5	42.0	45.1	39.6	-50.0	24.1	51.9	55.0
	hippo	LC	Decrease	23.9	45.2	28.7	28.5	48.7	32.0	36.1	40.3	-56.0	5.5	77.8	49.6
	kuhlii	LC	?	34.6	59.1	61.6	34.4	93.0	96.2	74.6	88.1	-7.8	94.6	129.0	99.8

Table S5.1 - Variation of occupied area throughout the 21st century in relation to area currently occupied by each species. See Fig. 5.3 for species abbreviations. Horizontal lines separate each biogeographic group.

Bioregion	Species	IUCN Status	Population Trend (2008)	2020				2050				2090			
				A1FI	A2	B1	B2	A1FI	A2	B1	B2	A1FI	A2	B1	B2
Mediterra-nean	lasiop	NT	Decrease	58.6	66.3	57.7	75.4	140.6	68.5	72.2	70.9	147.4	89.5	130.3	194.5
	mehe	Vu	Decrease	35.6	0.1	13.9	45.5	74.1	94.4	70.2	27.5	-54.8	67.5	97.6	95.5
	minio	NT	Decrease	24.2	22.4	55.8	21.5	49.5	38.7	70.6	60.9	-47.7	41.0	71.7	81.6
	myot	LC	Stable	28.0	32.7	47.6	45.3	51.7	22.5	34.4	47.7	-66.7	14.3	40.4	32.6
	savii	LC	Stable	107.5	75.0	87.7	68.8	188.9	158.0	130.7	139.9	110.5	171.4	195.3	229.0
	tada	LC	?	78.1	56.4	43.7	41.0	236.4	139.4	91.5	82.3	-5.3	148.3	199.9	222.2

Table S5.1 (cont.) - Variation of occupied area throughout the 21st century in relation to area currently occupied by each species. See Fig. 5.3 for species abbreviations. Horizontal lines separate each biogeographic group.

CHAPTER 6

General Discussion

"Science may be described as the art
of systematic over-simplification.
(...) good tests kill flawed theories;
we remain alive to guess again"
Karl Popper

6.1 Contribution of novel techniques for biodiversity management

The current rate of biodiversity loss urges for the development of methodologies that permit the fast and accurate development of conservation plans. For the management of threatened species, conservation biologists frequently rely on scarce and incomplete datasets (Barbosa *et al.* 2009). This is especially relevant for rare taxa where their rarity and difficulty of detection compromises the majority of statistical analyses (Chadès *et al.* 2008). Advances in several fields such as species distribution modelling and molecular analysis, offer the opportunity to produce quality results that would be more time- and resource- consuming when using more conventional methods. Moreover, by integrating those methodologies it is now possible to infer about the past, present and future status of populations or species with greater detail than ever (Cordellier & Pfenninger 2009). Yet, the reliability of these inferences still needs to be tested under a range of ecological conditions, geographical scales and time spans (Peterson *et al.* 2007; Jiménez-Valverde *et al.* 2008).

6.2 An integrative approach for bat conservation

In this thesis it was shown that novel species distribution modelling techniques may contribute significantly to conservation management by determining a

species' range and respective ecological predictors accurately. Moreover, by using distribution modelling it is possible to maximise survey efficiency by defining areas of probable occurrence. Even with a small and biased dataset, the known distribution of *B. barbastellus* in Portugal was extended greatly, and the importance of native woodland for the conservation of this bat was confirmed (Russo *et al.* 2004; Russo *et al.* 2005). It is of note however, that these goals were achieved during the timescale of this project (between 2005 and 2007), which was a considerably shorter time span compared with the collection of earlier data, gathered over *ca.* 20 years. In fact, since the discovery of the barbastelle in Portugal in 1980 up until the start of this work, less than 20 locations were known for the species. Several projects and thorough surveys, employing the most commonly used methods for bat surveys (mist-netting, acoustic surveys in areas defined by experts), were conducted over the last 20 years covering the majority of mainland Portugal (Rainho *et al.* 1998). On the other hand, upon completion of this thesis over 40 presence records were obtained for the same territory. In conjunction with genetic analysis, it was also possible to define priority areas for conservation of this species for the first time.

In the forthcoming future, results from this work will condensed into a few small documents that will be made available to Eurobats for an eventual online publication. These documents will focus on the guidelines on how to use predictive modelling to conduct surveys with rare species, as well as conservation recommendations for barbastelles in Europe. Finally, one document will also focus on the potential effect of climate change in European

bats and on suggestions on how to conduct local studies to develop suitable policies in response to this future threat.

6.3 Extending the applicability of an integrative approach

By combining palaeo-distribution models with genetic analysis, this thesis has shown that the southern European peninsulas of Iberia, Italy and the Balkans were the main glacial refugia for barbastelles during the Last Glacial Maximum. By using phylogeographic analyses it was possible to assess the relatedness of the different European populations of *B. barbastellus*, and thus determine the contribution of each glacial refugium to the postglacial colonisation process. Past distribution models placed phylogeographic inferences into a spatial context. Predicted population movements within glacial refugia gave a possible explanation for the high genetic diversity found in Iberia, and the lack of several intermediate haplotypes in Italy and the Balkans. Additionally, it was found that the Isle of Wight was probably close to a major colonisation route into mainland England. Despite its relative small area, a high genetic diversity was found in the isle, together with the presence of a unique ancestral haplotype.

The agreement between the locations of the glacial refugia predicted by both niche modelling and genetic analyses suggests that niche conservatism occurs in *B. barbastellus* (Waltari *et al.* 2007). The lack of, or slow, evolutionary rate of change in the species' niche supports the projection of current distribution models to other sets of ecological conditions. Such projections could include predictions of changes in distribution under climate change scenarios (Cordellier & Pfenninger 2009).

Future climate change will probably constitute one of the major threats to biodiversity (Parmesan & Yohe 2003). The associations between the geographic patterns of European bat species richness and climatic conditions had already been assessed by other authors (Ulrich *et al.* 2007). In this thesis the potential impact of climate change on the European bat fauna was investigated using climatic variables alone. It was demonstrated that species associated with colder climates will face greater challenges for survival. Nevertheless, if the current trend of planet warming persists, the more severe scenarios predict that even species associated with warmer climates may face several regional extinctions, or in some cases total extinction in Europe. In spite of these pessimistic predictions, models developed using climatic variables alone should be considered as "best case" scenarios (Pearson & Dawson 2003). Biotic interactions, such as competition or predation, were not taken into account, hence areas where suitable climatic conditions are predicted to subsist may not have ecological capacity to sustain populations by the lack of foraging habitats or suitable roosts. This work adds the European bat fauna to the extensive list of flora and fauna potentially threatened by climate change (Thuiller 2004; Araújo *et al.* 2006; Huntley *et al.* 2008).

Additionally, this work has highlighted the importance of taking into account species' biogeographic affinities when modelling the potential impact of climate change. The classification of bats into biogeographic groups allowed the detection of potential conservation problems that would probably be missed if analysing European bats as a whole. For example, Huntley *et al.* (2008) predicted that the number of European breeding birds per 50x50 km grid would decrease by 6.8 – 23.2% by the end of the century. By analysing bird

distributions as a single unit, these authors probably failed to detect more serious declines of some groups of species, especially for the groups associated to colder climates. In fact, the Boreal group included only four of the 28 studied species, though its predicted distribution shows that it is potentially the most threatened biogeographic group by climate change.

6.4 Final remarks

Despite several methodological and conservation advances resulting from this work, it would be possible to have widen the scope of the conclusions by increasing the amount of data and by incorporating more methodologies. For the chapters about barbastelles in Portugal, genetic analysis could have been improved by the inclusion of more samples and by including other genetic markers, like microsatellites, with a higher resolution that could determine each sex's contribution to population dispersal and fixation (Rossiter *et al.* 2001). Likewise, the inclusion of samples from European countries not covered in this study would allow a better understanding of *B. barbastellus* phylogeography, especially the localisation of postglacial routes. The rarity of the study species limited the distribution and availability of samples for genetic analyses. Nevertheless, some clear patterns could be inferred.

A multidisciplinary approach is clearly assuming a greater relevance in the fields of molecular biology, evolution, conservation biology and general ecology (e.g. Kidd & Ritchie 2006; Adora *et al* 2006). For instance, by integrating morphometry, predictive modelling and microsatellite analysis, it is possible to determine a species' evolutionary history and to spatially determine the location of vicariant features important in shaping population structure (Manel *et al.*

2003; Brito *et al.* 2008). Nevertheless, the integration of different techniques frequently produces complex outcomes, which are then associated with an ever existing scientific requirement of testing and validating all underlying methods and theories (Jiménez-Valverde *et al.* 2008).

In conclusion, this work has made a contribution to the validation of presence-only modelling techniques as an indispensible tool to determine the conservative distribution of a rare species. Moreover, by integrating genetic analysis it was possible to propose the first conservation measures for a species where there was almost no knowledge about its population structure, distribution and what were the ecological factors limiting its range. By expanding the geographical extent of this study, this thesis has also shown the relevance of species distribution modelling for predicting the distribution of a species in the past. The congruence between the results species distribution modelling with those from genetic analysis supports the use of modelling techniques to project models calculated from the current conditions to other sets of conditions. This is especially useful for other applications such as determining the impacts of invasive species (e.g. Jeschke & Strayer 2008), defining areas that may be suitable for the re-introduction of rare species (e.g. Hirzel *et al.* 2004) or determining the potential impact of climate change (e.g. Araújo *et al.* 2006). Indeed, this thesis approached this latter subject, by showing the potential impact of climate change on European bat diversity and by demonstrating the importance of considering the biogeographic affinities of a species when studying the predicted distribution of taxa.

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